

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 08-173195

(43)Date of publication of application : 09.07.1996

(51)Int.Cl.

C12Q 1/68

C12N 15/09

C12Q 1/70

(21)Application number : 07-268660

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(22)Date of filing : 17.10.1995

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(30)Priority

Priority number : 06265124 Priority date : 28.10.1994 Priority country : JP

(54) METHOD FOR DISCRIMINATING TYPES OF ENTEROVIRUS 71 TYPE AND COXSACKIE A GROUP VIRUS 16 TYPE AND DNA PROBE AND DNA FRAGMENT USED THEREFOR

(57)Abstract:

PURPOSE: To enable to discriminate the types of the Enterovirus 71 type (EV 71) and the Coxsackie A group virus 16 type (CA 16) in high accuracy.

CONSTITUTION: The method for discriminating the types of the EV 71 and the CA 16 comprises (i) multiplying a DNA sequence coding for a part of the 5'-nontranslating regions of the EV 71 and the CA 16 and a part of Vp4 and Vp2 proteins having DNA sequences specific to the serum types of the viruses, (ii) determining the DNA sequence of the DNA region coding for the Vp4 protein in the multiplied DNA, (iii) designing DNA sequences complementary to the DNA sequences on the basis of DNA sequences specific to the serum types of the EV 71 and the CA 16 in the DNA sequences, respectively, and subsequently (iv) analyzing an ability for combining the obtained DNA probes with the multiplied DNA obtained by the method (i).

LEGAL STATUS

[Date of request for examination] 25.06.2002

[Date of sending the examiner's decision of rejection] 20.12.2005

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

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CLAIMS

[Claim(s)]

[Claim 1] (i) Enterovirus 71 mold and Cocksackie A group virus 16 mold, The DNA field which carries out the code of some ****4 and ****2 proteins which have a specific DNA array in each human serum protein type of a part of 5'untranslation region and enterovirus is amplified. (ii) The DNA array of the DNA field which carries out the code of the ****4 protein under this magnification DNA is determined. It is based on a DNA array specific to each human serum protein type of the enterovirus 71 mold under this DNA array, and Cocksackie A group virus 16 mold. (iii) Design the DNA array which has a complementarity respectively in this DNA array, respectively, and it is made into a DNA probe. (iv) The mold differentiation approach of the enterovirus 71 mold characterized by analyzing a binding affinity with the magnification DNA obtained by the approach of this DNA probe and the above (i), and Cocksackie A group virus 16 mold.

[Claim 2] The DNA array which has the DNA field which carries out the code of the ****4 protein of the enterovirus 71 mold under magnification DNA, and a complementarity It is the DNA array shown by the array number 31, the array number 33, the array number 35, the array number 37, or the array number 41. The approach according to claim 1 the DNA array which has the DNA field which carries out the code of the ****4 protein of the Cocksackie A group virus 16 mold under magnification DNA, and a complementarity is a DNA array shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, or the array number 40.

[Claim 3] The DNA probe which has the magnification DNA including the DNA array shown by the array number 31, the array number 33, the array number 35, the array number 37, or the array number 41 of the DNA field which carries out the code of the ****4 protein of enterovirus 71 mold, and a complementarity.

[Claim 4] The DNA probe which has the magnification DNA including the DNA array shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, or the array number 40 of the DNA field which carries out the code of the ****4 protein of Cocksackie A group virus 16 mold, and a complementarity.

[Claim 5] The DNA fragment which is ****4 protein of enterovirus 71 mold and carries out the code of the protein including the amino acid sequence shown by the array number 1 in the amino acid sequence.

[Claim 6] The DNA fragment which is ****4 protein of Cocksackie A group virus 16 mold, and carries out the code of the protein including the amino acid sequence shown by the array number 2 in the amino acid sequence.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[The technical field to which invention belongs] This invention relates to the base sequence in the differentiation approach of the enterovirus 71 mold (this may be written as "EV71" below) belonging to enterovirus, and Coxsackie A group virus 16 mold (this may be written as "CA16" below), the DNA probe used for it, and the gene field which carries out the code of the ****4 protein of enterovirus 71 mold and Coxsackie A group virus 16 mold to a list.

[0002]

[Description of the Prior Art] In order to classify the enterovirus (Enterovirus) belonging to the Picornaviridae (Picornaviridae) into about 70 kinds of human serum protein types and to show a variegated infectious disease, it is difficult to presume the virus which becomes a cause from a clinical manifestation. Therefore, separation identification of a virus is needed for deciding a pathogen. However, a current enterovirus separation method of identification separates a virus using cultivation, and the protection test is still more nearly required for it for identification. And two - four weeks is required for the isolation culture of these viruses. Furthermore, in the protection test which used the neutralization antiserum of a standard stock, the separation stock in which human serum protein type differentiation is impossible appears frequently. This is considered for the gene of enterovirus to vary extremely in a nature at high speed, and production of the antiserum which always neutralizes a fresh separation stock is needed for these solutions.

[0003] High sensitivity and polymerase chain reaction method [Polymerase Chain Reaction which amplifies DNA specifically Law, ; which writes this as the "PCR method" below -- Saiki and others, Science, 230 volumes, p.1350-1354, and 1985 After year reference] is developed, enterovirus The PCR method using a primer complementary to the base sequence of 5'untranslation region, 5' -- in an untranslation region ****4 and ****2 protein To the base sequence of the gene field which carries out a code, a complementarity The primer which it has [Rotbart and H. which are detected by the used PCR method, S.J.Clinical Microbiology, 28, and 438-442 (1990); Olive and D.M. and S.J.General Virology, 71, 2141-2147(1990)].

[0004] Furthermore, mold identification of the enterovirus by the stringent reverse solid phase hybridization method using DNA amplified by PCR is reported [clinical [, such as **** Hiroaki,], a virus, 22 volumes, and page 199-207 (1994)]. However, in this experiment, the reactivity of a target gene and a probe changes with differences in an age or an area also in the same human serum protein type, and it is expected to be difficult to select a specific viral strain to a probe. In the disease by enterovirus, since the virus separated from the patient occupies 90% or more by EV71 and CA16, hand, foot and mouth disease can identify the epidemia virus of hand, foot and mouth disease by detecting quickly and simply two sorts of human serum protein types.

[0005]

[Problem(s) to be Solved by the Invention] This invention aims at offer of the approach of carrying out mold differentiation of EV71 and CA16 in a high precision.

[0006]

[Means for Solving the Problem] As a result of repeating examination wholeheartedly that the above-mentioned purpose should be attained, in the base sequence of the gene field which carries out the code of the ****4 protein of EV71 and CA16, this invention persons had a specific base sequence in each human serum protein type, find out that EV71 and CA16 can discriminate in a high precision using this base sequence, and came to complete this invention. That is, this invention provides following 1-6 with the DNA probe and DNA fragment which are used for the mold differentiation approach of the enterovirus 71 mold of a publication, and Cocksackie A group virus 16 mold, and it.

[0007] 1. (i) enterovirus 71 mold and Cocksackie A group virus 16 mold, The DNA field which carries out the code of some ****4 and ****2 proteins which have a specific DNA array in a part of 5'untranslation region and the human serum protein type of enterovirus is amplified. (ii) The DNA array of the DNA field which carries out the code of the ****4 protein under this magnification DNA is determined. It is based on a DNA array specific to each human serum protein type of the enterovirus 71 mold under this DNA array, and Cocksackie A group virus 16 mold. (iii) Design the DNA array which has a complementarity respectively in this DNA array, respectively, and it is made into a DNA probe. (iv) The mold differentiation approach of the enterovirus 71 mold characterized by analyzing a binding affinity with the magnification DNA obtained by the approach of this DNA probe and the above (i), and Cocksackie A group virus 16 mold.

[0008] 2. DNA Array Which Has DNA Field Which Carries Out Code of the ****4 Protein of Enterovirus 71 Mold under Magnification DNA, and Complementarity It is the DNA array shown by the array number 31, the array number 33, the array number 35, the array number 37, or the array number 41. The approach of one above-mentioned publication that the DNA array which has the gene field which carries out the code of the ****4 protein of the Cocksackie A group virus 16 mold under magnification DNA, and a complementarity is a DNA array shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, or the array number 40.

3. DNA probe which has magnification DNA including DNA array shown by array number 31, array number 33, array number 35, array number 37, or array number 41 of DNA field which carries out code of ****4 protein of enterovirus 71 mold, and complementarity.

[0009] 4. DNA probe which has magnification DNA including DNA array shown by array number 32, array number 34, array number 36, array number 38, array number 39, or array number 40 of DNA field which carries out code of ****4 protein of Cocksackie A group virus 16 mold, and complementarity. 5. DNA fragment which is ****4 protein of enterovirus 71 mold and carries out code of protein including amino acid sequence shown by array number 1 in the amino acid sequence.

6. DNA fragment which is ****4 protein of Cocksackie A group virus 16 mold, and carries out code of protein including amino acid sequence shown by array number 2 in the amino acid sequence. Hereafter, it explains for details further about this invention.

[0010] Especially in operation of this invention, unless it is directed, the conventional technique in the molecular biology in technical within the limits of the field concerned, microbiology, a recombinant DNA, and immunology is adopted. Such technique is explained in detail into reference. For example, please refer to the following reference. Maniatis, Fitch, and separate volume (1991); volume for University of Tokyo Institute of Medical Science carcinostatic research sections, and cell technology experiment protocol . Sambrook, MOLECULAR CLONING (1991); A LABORATORY MANUAL (1982); Kevin struhl and others, CURRENT PROTOCOLS IN MORECULAR BIOLOGY, one volume, and two volumes; the volume for Masami Muramatsu, lab manual gene engineering (1990) ; Masami Muramatsu The volume for Hiroto Okayama, a gene engineering handbook, experimental medicine

[0011] 1. Enterovirus 71 Mold And a part of 5'untranslation region of Cocksackie A group virus 16 mold A specific base sequence to the human serum protein type of enterovirus Some ****4 and ****2 proteins which it has a code -- carrying out -- a gene -- a field -- magnification -- enterovirus -- 71 -- a mold -- and -- Cocksackie -- A -- a group -- a virus -- 16 -- a mold -- five -- ' -- an untranslation region -- a part -- enterovirus -- a human serum protein type -- being specific -- a base sequence -- having -- **** -- four -- and -- **** -- two -- protein -- a part -- a code -- carrying out -- a gene -- a field (below this)

Magnification which may be called for short "a gene field including the human serum protein type specific base sequence of enterovirus" can be performed as follows.

[0012] First, the enterovirus isolation culture stock of known [human serum protein type] and the human serum protein type by which subculture is carried out extract RNA from a known enterovirus standard stock etc. with a conventional method, and produces cDNA from this extract RNA using reverse transcriptase. The die length which includes the gene field which carries out the code of 5'untranslation region of enterovirus 71 mold and Cocksackie A group virus 16 mold, and ****4 and ****2 for the oligonucleotide which has a complementarity as a primer in the mold intersection of the upstream of the gene field which includes the human serum protein type specific base sequence of enterovirus in this cDNA, and a down-stream mold intersection amplifies the gene DNA field of about 650 bases.

[0013] the PCR method for which magnification of a gene is usually used -- [-- JP,61-274697,A, JP,62-281,A, 239 Sakai sScience(s), and p.487-491 reference] can perform the detail of this PCR method easily. As long as it uses for coincidence the oligonucleotide which has a complementarity in the mold intersection of the upstream of a gene field including a human serum protein type specific base sequence, and a down-stream mold intersection as an oligonucleotide (this may be called for short a "human serum protein type common primer" below) which can be used as a primer on the occasion of magnification of a gene field including the human serum protein type specific base sequence of enterovirus, you may be what kind of oligonucleotide. It is appropriate to use as a primer the oligonucleotide which was specific to enterovirus, and set the high base sequence of similarity as 5'untranslation region (upstream mold intersection) and ****2 field (down-stream mold intersection) between seeds, and carried out chemosynthesis in them based on the base sequence based on desirable known human serum protein type specific base sequence data.

[0014] As an example of the above-mentioned primer, they are D.M.Olive et al, 71 Journal of General Virology, and a page. 2141-2147 (1990) The oligonucleotide shown by the array number 29 and the array number 30 which are indicated by the oligonucleotide shown by the array number 27 and the array number 28 which are indicated, and the JP,6-311900,A specification which invention-in-this-application persons proposed previously can be mentioned. the inside of these primers -- 5' -- the oligonucleotide shown by the array number 29 as a primer by the side of an end, and 3' -- it is appropriate to use for coincidence the oligonucleotide shown by the array number 28 as a primer by the side of an end.

[0015] Chemosynthesis of the above-mentioned primer can be easily carried out in itself with the solid phase synthesis method using a model 381-ADNA composition machine, known the nucleic-acid-biosynthesis equipment usually used, for example, applied biotechnology systems (Applied Biosystems) company make, etc. It can dissociate with a conventional method, for example, agarose gel electrophoresis, and the above-mentioned magnification DNA can be detected as a DNA band, and, thereby, can check the gene DNA of the enterovirus 71 mold and Cocksackie A group virus 16 mold origin.

[0016] 2. The base sequence of the decision above-mentioned magnification DNA of the base sequence of the gene field which carries out the code of the ****4 protein under magnification DNA is for example, applied biotechnology systems company make and model 373-A. An auto sequencer is used and it is Dye DeoxyTM. It can determine by the terminator method. The base sequence of the gene field which carries out the code of the ****4 protein can be determined by searching for the base sequence [a protein nucleic-acid enzyme, 137 volumes, No. 14, and page 2609-2618 (1992) reference] corresponding to the amino acid sequence which cuts ****4 known protein and ****2 known protein of the translation initiation codon in the determined base sequence, and enterovirus.

[0017] The array of the gene field which carries out the code of the ****4 protein of EV71 obtained in this way is shown in the array number 3 - the array number 21, and the combination of the amino acid sequence is shown in the array number 1. Moreover, the array of the gene field which carries out the code of the ****4 protein of CA16 is shown in the array number 22 - the array number 26, and the combination of the amino acid sequence is shown in the array number 2. The DNA fragment of this invention which includes the above-mentioned base sequence is the DNA synthesizer usually used only

not only in cDNA compounded from RNA of EV71 and CA16 separation stock, for example, the above-mentioned applied biotechnology systems company make, and model 381-A. It may be compounded using a DNA synthesis machine.

[0018] 3. The base sequence of the DNA probe (this may be called a "EV71 unique probe" for short below) which has a complementarity in the specific base sequence of the design and synthetic enterovirus 71 mold of a DNA probe can be designed by analyzing a specific array to EV71 under array shown by said array number 3 - the array number 21. Similarly, the base sequence of the DNA probe (this may be called a "CA16 unique probe" for short below) which has a complementarity in the specific base sequence of Coxsackie A group virus 16 mold can be designed by analyzing a specific array to CA16 under array shown by said array number 22 - the array number 26.

[0019] As an EV71 unique probe obtained in this way, the oligonucleotide shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, or the array number 40 can be mentioned, for example, for example as the oligonucleotide shown by the array number 31, the array number 33, the array number 35, the array number 37, or the array number 41, and a CA16 unique probe. The oligonucleotide shown as an EV71 unique probe by the array number 32, the array number 39, and the array number 40 as the array number 31 and the array number 41, and a CA16 unique probe can be preferably mentioned among the above-mentioned human serum protein type unique probes. The above-mentioned DNA probe is model 381-A, known the nucleic-acid-biosynthesis equipment usually used, for example, applied biotechnology systems company make, in itself. With the solid phase synthesis method using a DNA synthesis machine etc., chemosynthesis can be carried out easily.

[0020] 4. EV71 and CA16 can be judged using a human serum protein type [in which the enterovirus 71 mold and the coxsackie virus 16 mold carried out the mold differentiation above] unique probe as follows. First, viral RNA is extracted from the virus isolation culture stock from the clinical specimen extracted at the time of a medical examination with a conventional method. The gene field which carries out the code of some 5'untranslation region [of enterovirus / a part of], ****4, and ****2 proteins after compounding cDNA using said human serum protein type common primer from this extract RNA is amplified by said PCR method. Detection of Magnification DNA can separate Magnification DNA by agarose electrophoresis, can be made to be able to color it by UV irradiation etc. after dyeing with a suitable stain, for example, the ethidium bromide etc., and can be performed by checking the DNA band.

[0021] For the DNA fragment amplified using said human serum protein type common primer, and detection of said human serum protein type unique DNA probe 71, i.e., EV, mold differentiation of EV71 and CA16 The array shown by the array number 31, the array number 33, the array number 35, the array number 37, and the array number 41 again for example, for detection of CA16 For example, the DNA probe produced by carrying out the indicator of the oligonucleotide which has the array shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, and the array number 40 It can be made to be able to hybridize with a conventional method on solid phase, and the class of human serum protein type unique DNA probe can be performed by carrying out detection analysis.

[0022] Although association of DNA to a solid phase top can be performed by carrying out the chemical bond of the approach DNA, for example, the magnification, usually using known in itself with a conventional method to suitable solid phase, for example, a nylon membrane, after denaturalizing The approach (this may be called the "Southern-blotting method" for short below) of neutralizing the above-mentioned agarose gel preferably used in order to check Magnification DNA after denaturalizing, for example, fixing to a nylon membrane after transferring Magnification DNA is mentioned.

[0023] The phosphorylation of 5'end can perform the indicator of a DNA probe using [gamma-32P] ATP and T-four polynucleotide kinase (Toyobo Co., Ltd. make). Moreover, detection of an indicator object can perform the autoradiography of the nylon membrane which performed hybridization with the DNA probe by which the indicator was carried out, and can be performed by investigating the existence of hybridization with each DNA probe.

[0024] Examples, such as a DNA fragment which can be amplified, are shown in drawing 17 using the field which carries out the code of some a part of gene field including above-mentioned human serum protein type specific DNA array of enterovirus used by this invention, i.e., 5'untranslation region of enterovirus, ****4, and ****2 proteins, a human serum protein type common primer, a DNA probe, and this primer. As for the location (base pair number) of the gene field where double digits carry out the code of an array number and the ****4 protein with which a DNA probe combines the figure in (), the magnitude of the DNA fragment with which the figure in [] is amplified, and bp, a base pair is shown among drawing.

[0025]

[Example] Next, although an example is given and this invention is further explained to a detail, the following example is given only as an aid which acquires recognition concrete about this invention, and the range of this invention is not limited at all by this.

[0026] Example 1 It experimented using the enterovirus separation stock and standard stock with which it dissociated from the patient of (Sequence A) use microorganism following of EV71 and CA16 isolation-culture stock, and the human serum protein type was identified by the protection test using the specific antiserum. The standard stock used here is a virus standard stock by which subculture is carried out in the National Institute of Health.

[0027]

(1) Enterovirus separation stock Stock name (human serum protein type) Separation stage Coxsackie A group virus 16 mold (CA16)

1547/79 1979 4057/81 1981 0216/86 1986 0241/91 1991 Enterovirus 71 mold (EV71)

NAGOYA/70 1970 0108/78 1978 3059/78 1978 3359/83 1983 4132/85 1985 T86236a / 861986 years 1091/89 1989 year 0445 / 90 1990 0004/78 1978 year 1096 / 86 1986 0872/89 1989 year 2587 / 89 1989 2603/89 1989 year 0375 / 90 1990 2136/90 1990 year 2398 / 90 1990 4094 / 90 1990 0419/90 1990

[0028] (2) Standard **** name (human serum protein type) Separation stage Coxsackie A group virus 16 molds (CA16)

Enterovirus 71 molds (EV71)

[0029] (B) From the extract above-mentioned virus liquid of RNA, the SUMAI test R kit (Sumitomo Metal, Ltd. make) extracted RNA, and isopropanol precipitation was performed.

(C) cDNA which originates in each virus using a reverse transcriptase (product made from GIBCO/BRL) by using as mold RNA obtained by the synthetic above-mentioned (B) term of cDNA was compounded.

(D) the oligonucleotide shown by the synthetic array number 28 and the array number 29 of a primer for PCR -- phospho friend DAIDO (Phosphoamidide) -- law -- applied biotechnology systems company make and model 381-A It compounded using the DNA synthesis machine. The above-mentioned oligonucleotide was refined using the OPCTM cartridge, and it was used as a primer of PCR.

[0030] (E) Target's DNA magnification (PCR method)

Reaction mixture is 10mM(s) about final concentration. Tris-HCl (pH8.0), 50mM(s) KCl, 0.001% gelatin, 0.55mM MgCl₂, 0.05mM(s) In dNTPs and the solution made into the formamide 2.5% Above-mentioned oligonucleotide composition primer (array number 28 and array number 29; every 50microM) every 0.1microl, enterovirus cDNA compounded by the aforementioned (C) term 100ng-1microg and Taq polymerase (made in Japanese Roche) 0.125microl (0.625U) -- adding -- total 50microl ** -- what was carried out was prepared. PCR is 95-degree-C 30 seconds, and 55-degree-C reaction condition for 30 seconds, and amplified Target DNA using the amplification system (Amplification System; SHITASU) in 14 cycle.

[0031] Furthermore, they are 10mM(s) about final concentration to the above-mentioned solution. Tris-HCl (pH8.0), 50mM(s) KCl, 0.001% gelatin, 2.35mM MgCl₂, 0.15mM(s) In dNTPs and the solution made into the formamide 2.5% Above-mentioned oligonucleotide composition primer (array number 28 and array number 29; every 50microM) every 1.0microl, Total 50microl which added Taq polymerase 0.25microl (1.25U) The solution was added and it amplified in 95-degree-C 30-second and 55-degree-C 30 seconds, and 72-degree-C 45 seconds and 40 cycle.

[0032] (F) The ethidium bromide was added to agarose gel of 3.0% of checks of the magnification DNA by gel electrophoresis ml 0.5microg /, and electrophoresis of DNA amplified by the above-mentioned (E) term was performed. 254nm ultraviolet rays were irradiated after migration, the coloring reaction of the ethidium bromide detected the DNA band, and the target DNA band of about 650 bases originating in the gene field which carries out the code of a part of 5'untranslation region of enterovirus and some ****4 and ****2 proteins was detected.

[0033] (G) Centricon-100 (Amicon make) is used for the PCR product checked by the decision above (F) of the base sequence of ****4 field, and it is after purification and Dye DeoxyTM. The terminator method Cycle Sequencing It reacts and is model by applied biotechnology systems company 373-A. The base sequence was determined using the DNA sequencer. By searching for the base sequence corresponding to the amino acid sequence which cuts ****4 known protein and ****2 known protein of the translation initiation codon in the determined base sequence, and enterovirus, the base sequence of the gene field which carries out the code of the ****4 protein was determined. The array which carries out the code of the ****4 protein of CA16 for the array which carries out the code of the ****4 protein of EV71 obtained in this way to the array number 3 - the array number 21 is shown in the array number 22 - the array number 26.

[0034] Example 2 It experimented using the 39 following kinds of human serum protein type enterovirus standard stocks by which subculture is carried out in the mold (differentiation A) use microorganism (1) standard stock National Institute of Health of EV71 and CA16. Each of such enteroviruses is standard stocks with which the human serum protein type is identified by the protection test which used the specific antiserum.

[0035]

A stock name (human serum protein type) A cable address The Coxsackie A group virus 2 molds CA2 ** 4** CA4 ** 6** CA6 ** 7**CA7 ** 9** CA9 ** 10 ** CA10 ** 16 ** CA16 **24** CA24 The Coxsackie B group virus 1 mold CB1 **2** CB2 ** 3** CB3** 4** CB4 ** 5** CB5 **6** CB6 Echovirus 1 mold E1 **3 ** E3 ** 4** E4 ** 5** E5 ** 6** E6 ** 7** E7 ** 9** E9 ** 11 ** E11 ** 12** E12** 13** E13 ** 14** E14** 16**E16 ** 17** E17 **18** E18 ** 19 ** E19 **21** E21** 22** E22 ** 24** E24 ** 25 ** E25 ** 30** E30 Enterovirus 70 mold EV70 ** 71 ** EV71 Poliovirus 1 mold PV1 ** 2 ** PV2 ** 3 ** PV3 [0036] (2) It experimented using the enterovirus separation stock with which it dissociated from the patient of the enterovirus separation stock following, and the human serum protein type was identified by the protection test using the specific antiserum.

[0037]

Stock name Separation stage Coxsackie A group virus 16 mold (CA16)

4057/81 1981 0843/87 1987 0071/88 1988 1045/90 1990 1051/90 1990 0241/90 1990 2500/92 1992 enterovirus 71 mold (EV71)

NAGOYA/70 1970 2105/73 1973 2089/73 1973 2146/73 1973 2203/73 1973 2377/73 1973 2381/73 In 1973 0108/78 1978 3059/78 In 1978 0193/78 1978 0297/78 In 1978 0333/78 1978 0761/82 1982 year FE-27 1982 0272/83 1983 year FE-18 1983 FE-20 1983 4132/85 1985 0866/86 1986 0891/86 In 1986 0948/86 1986 1025/86 In 1986 1027/86 1986 1079/86 1986 1096/86 1986 1163/86 1986 0460/87 1987 0443/88 1988 2587/89 1989 2603 / 891989 years 2136/90 1990 2234/90 1990 2398/90 1990 0419/90 1990 0445/90 1990 0189 / 901990 years 4094/90 1990 FE-45 1990 year FE-46 1990 T91-Y563 1991 1264/92 1992 2326 / 921992 years 2909/93 1993 0089/93 1993 0133/93 1993 0136/93 1993 0146/93 1993 2200/93 1993 year 0154 / 93 1993 0159/93 1993 2122/93 1993 2167/93 1993 2168/93 1993 2173/93 1993 0565/93 1993 0630/93 In 1993 0679/93 1993 0702/93 In 1993 0724/93 1993 0748/93 In 1993 0774/93 1993 0791/93 1993 year FE-57 1993 T93-128 1993 year T93-133 1993 T93-Y998 1993

[0038] (B) As (B) - (F) term of an RNA extract, cDNA composition and magnification by PCR of an enterovirus gene, and its detection example 1 showed, RNA of the above-mentioned enterovirus standard stock and a separation stock was extracted, and magnification was checked in the electrophoresis using agarose gel after amplifying the part corresponding to a part of 5'untranslation region and some ****4 and ****2 proteins. In addition, it is HaeIII of phix174 phage as a marker. The decomposition product was migrated to coincidence.

[0039] (C) The agarose gel used by the (B) term of the Southern blot technique example 2 for the check of Magnification DNA was put in for 30 minutes for 30 minutes with alkali denaturation liquid (0.5M NaOH, 1.5M NaCl) after processing and with neutralization liquid (1 M Tris-HCl, 1.5M NaCl), gel was returned to neutrality, and DNA was denatured. Furthermore, DNA which performed Southern blotting overnight and shifted to the nylon membrane with the conventional method was made to fix by 254nm 1.2x10⁵micro JOULES/cm² (UV stratalinkerTM; Stratagene make) of ultraviolet rays.

[0040] (D) The base sequence [the array number 26 from the array number 3] of the DNA field which carries out the code of the ****4 protein of CA16 and EV71 shown by a design and the synthetic example 1 (G) term of a human serum protein type specific probe was analyzed, the array number 31 and the array number 41, and the CA16 unique probe were designed like the array number 32, the array number 39, and the array number 40, and the EV71 unique-from these arrays probe compounded according to the example 1 (D) term. The phosphorylation of 5'end performed the indicator of the above-mentioned DNA probe using [gamma-32P] ATP and T-four polynucleotide kinase (Toyobo Co., Ltd. make).

[0041] (E) The membrane produced by the hybridization above-mentioned example 2 (C) term is 1.0M NaCl and 50mM as reaction mixture. Tris-HCl (pH7.5), 0.5%PVP, 0.2% heparin, 1mM It processed at 50 degrees C for 1 hour using EDTA and 2%SDS, and pre hybridization was performed. Hybridization added the [gamma-32P] ATP indicator DNA probe compounded by the (D) term of an example 2 105 cpm/ml to the above-mentioned hybridization solution, and made it react to it 50 degrees C for 4 hours. It washed at 50 degrees C in the 5xSSC-0.1%SDS solution, the membrane was washed twice at 50 degrees C in the 2xSSC-0.1%SDS solution after 5 minutes and 2 times washing for 5 minutes, and the probe combined nonspecific was removed. After drying a nylon membrane, on the lap, after the room temperature performed a package and autoradiography for 15 minutes further for 45 minutes at -80 degrees C, they were developed.

[0042] Formation of the hybrid of the CA16 specific DNA probe (the array number 32, the array number 38, and array number 39) and Magnification DNA which were obtained in this way is shown in drawing 1 - drawing 8, and the hybridization of an EV71 specific DNA probe (the array number 31 and array number 41) and Magnification DNA is shown in drawing 9 - drawing 16. The passage clear from drawing, all EV71 separation stocks react to an EV71 specific DNA probe, and all CA16 separation stocks react to a CA16 specific DNA probe, and there is no cross reaction between a human serum protein type specific DNA probe and DNA obtained from the enterovirus standard stock of each human serum protein type, and it became clear that this was specific association. According to the approach of this invention the above-mentioned passage, CA16 and EV71 can discriminate with high precision.

[0043]

[Layout Table]

array number: -- die-length [of one array]: -- mold [of 69 arrays]: -- amino acid topology: -- Ggg of under the information following array of straight chain-like others, and the 3rd amino acid number Ser Or Thr being shown -- Hhh of the 7th amino acid number Ala Or Thr It is shown and is the 9th amino acid number. Iii Arg or -- Xaa -- being shown -- Jjj of the 17th amino acid number Asn or -- Xaa -- being shown -- Kkk of the 31st amino acid number Asn Or Xaa being shown -- Lll of the 34th amino acid number Lys Or Xaa being shown -- Mmm of the 35th amino acid number Asp Or Xaa being shown -- Nnn of the 43rd amino acid number Lys Or Xaa being shown -- Ooo of the 47th amino acid number Lys or Xaa being shown -- Ppp of the 49th amino acid number Asp or Xaa It is shown and is Qqq of the 53rd amino acid number. Phe or Xaa It is shown and is Rrr of the 54th amino acid number. Ala Or Gly Or Xaa It is shown and is Sss of the 55th amino acid number. Asn Or Xaa It is shown.

Array Met Gly Ggg Gln Val Ser Hhh Gln Iii Ser Gly Ser His Glu Asn Ser 5 10 15 Jjj Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Kkk Tyr 20 25 30 TyrLll Mmm Ser Tyr Ala Ala Thr Ala Gly Nnn Gln Ser Leu Ooo Gln 35 40 45 Ppp Pro Asp Lys Qqq Rrr Sss Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60Ala Ala Pro Leu Lys 65 [0044] array number: -- die-length [of two arrays]: -- mold [of 69

arrays]: -- amino acid topology: -- Aaa of under the information following array of straight chain-like others, and the 9th amino acid number Arg Or Xaa being shown -- Bbb of the 19th amino acid number

Ala Or Xaa being shown -- Ccc of the 21st amino acid number Glu Or Xaa It is shown and is Ddd of the 23rd amino acid number. Ser Or Thr It is shown and is Eee of the 24th amino acid number. Thr Or Xaa It is shown and Fff of the 29th amino acid number is Thr. Or Pro It is shown.

Array Met Gly Ser Gln Val Ser Thr Gln Aaa Ser Gly Ser His Glu Asn Ser 5 10 15 Asn Ser Bbb Ser Ccc Gly Ddd Eee Ile Asn Tyr Thr Fff Ile Asn Tyr 20 25 30 TyrLys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln 35 40 45 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 Ala Pro ProLeu Lys 65 [0045] array number: -- die-length [of three arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus enterovirus type name: -- the description description of a BrCr array The notation:CDS existence location to express : 1 - 207 description The 1-207th base sequence array ATG GGC TCC CAG GTC TCC ACA CAG CGA TCC GGC TCG CAT GAG AAT TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 1015 AAC TCA GCC ACG GAA GGC TCC ACT ATA AAT TAC ACA ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAA GAC TCG TAT GCT GCC ACT GCT GGA AAG CAA AGT CTC AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCT GAC AAG TTT GCG AAC CCT GTG AAG GAC ATC TTT ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys AspIle Phe Thr Glu Met 50 55 60 GCA GCG CCC TTA AAG 207Ala AlaPro Leu Lys 65 [0046] array number: -- die-length [of four arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of NAGOYA/70 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT ACT CAG CGG TCC GGC TCC CAC GAG AAT TCC of the gene field which carries out the code of the ****4 determined protein of information en TEROUISURU71 mold besides approach:E 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 1015 AAT TCA GCT ACA GAA GGT TCC ACC ATT AAT TAC ACT ACT ATC AAT TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCA GGC AAG CAG AGC CTC AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCT GAC AAG TTT GGC AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Gly Asn Pro Val Lys AspIle Phe Thr Glu Met 50 55 60 GCG GCG CCA CTG AAG 207Ala Ala Pro Leu Lys 65 [0047] array number: -- die-length [of five arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 0108/78 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT ACT CAG CGG TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAT TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATC AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCAGGT AAG CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser LeuLys Gln 35 40 45 GAC CCT GAC AAG TTT GCTAAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCC GCG CCA CTG AAG 207Ala Ala Pro Leu Lys65 [0048] array number: -- die-length [of six arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- enterovirus type 71-share name: -- the description description of 3059/73 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence arrays ATG of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E GGT TCA CAA GTG TCT ACT CAG CGG TCC GGC TCC CAC GAG AAT TCT 48 Met Gly Ser Gln Val

Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC
ATT AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr
Ile Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCA GGC AAG CAG AGC CTT
AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCT
GAC AAG TTT GCT AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys
Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCC GCG CCA CTG AAG 207 Ala Ala
Pro Leu Lys 65 [0049] array number: -- die-length [of seven arrays]; -- mold [of 207 arrays]; --
number [of nucleic-acid chains]; -- single-strand topology: -- class [of straight chain-like array]; --
cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: --
notation:CDS existence location: showing the description description of 0004/78 array -- the 1-207th
base sequences of the gene field which carries out the code of the ****4 protein of information
enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGA TCG
CAG GTG TCC ACA CAA CGC TCT GGT TCG CAT GAA AAT TCT 48 Met Gly Ser Gln Val Ser
Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCC ACT GAA GGT TCC ACT ATA
AAC TAC ACC ACC ATC AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile
Asn Tyr 20 25 30 TAT AAG GAC TCT TAT GCC GCT ACA GCA GGC AAA CAG AGC CTT AAG
CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA
GAC AAG TTT GCA AAT CCC GTT AAA GAT ATT TTC ACT GAG ATG 192 Asp Pro Asp Lys
Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCG GCA CCA CTG AAA 207 Ala Ala
Pro Leu Lys 65 [0050] array number: -- die-length [of eight arrays]; -- mold [of 207 arrays]; --
number [of nucleic-acid chains]; -- single strand topology: -- class [of straight chain-like array]; --
cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the
description description of 3359/83 array The notation:CDS existence location to express : 1 -207
description The 1-207th base sequence array ATG GGT TCA CAA GTA TCC ACT CAG CGG TCC
GGC TCC of the gene field which carries out the code of the ****4 determined protein of information
enterovirus 71 mold besides approach:E CAC GAG AAT TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg
Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC
ACT ACT ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25
30 TAC AAG GAC TCT TAT GCT GCC ACA GCA GGC AAA CAG AGCCTC AAA CAA 144 Tyr
Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCC GAC AAG TTT
GCT AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro
Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCG GCG CCG CTGAAG 207 Ala Ala Pro Leu Lys 65
[0051] array number: -- die-length [of nine arrays]; -- mold [of 207 arrays]; -- number [of nucleic-
acid chains]; -- single-strand topology: -- class [of straight chain-like array]; -- cDNA to genomic
RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence
location: showing the description description of 4132/85 array -- the 1-207th base sequences of the gene
field which carries out the code of the ****4 protein of information enterovirus 71 mold besides
approach:E which determined 1 -207 description Array ATG GGC TCA CAA GTG TCT ACT CAG
CGA TCC GGC TCC CAC GAG AAT TCC 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His
Glu Asn Ser 5 10 15 ANT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC
ANC TAT 96 Xaa Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Xaa Tyr 20 25 30 TAC ANA
GNC TCT TAT GCT GCA ACA GCA GGC ANA CAG AGY CTT AMA CAA 144 Tyr Xaa Xaa Ser
Tyr Ala Ala Thr Ala Gly Xaa Gln Ser Leu Xaa Gln 35 40 45 GMC CCT GAT AAG TKT GCT AMT
CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Xaa Pro Asp Lys Xaa Ala Xaa Pro Val Lys Asp
Ile Phe Thr Glu Met 50 55 60 GCC GCG CCA CTA AAA 207 Ala Ala Pro Leu Lys 65 [0052] array
number: -- die-length [of ten arrays]; -- mold [of 207 arrays]; -- number [of nucleic-acid chains]; --
single strand topology: -- class [of straight chain-like array]; -- cDNA to genomic RNA origin living
thing name: -- 71 shares of enterovirus type name: -- the description description of a T86236a array The
notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG
GGT ACA CAA GTA TCC ACT CAG SGG TCC GGC TCC of the gene field which carries out the
code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC

GAG AAT TCT 48 Met Gly Thr Gln Val Ser Thr Gln Xaa Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAC TAC ACT ACT ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCTTAT GCT GCT ACA GCAGGC AAA CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCC GAC AAG TTT GCTAAT CCT GTC AAG GAC ATTTTT ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCG GCG CCA CTG AAG207Ala AlaPro Leu Lys 65 [0053] array number: -- die-length [of 11 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 1096/86 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGC TCC ACC ATA AAC TAT ACT ACC ATC AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT GCC GCC ACA GCA GGC AAA CAG AGC CTT AAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCG AAT CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys65 [0054] array number: -- die-length [of 12 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 1091/89 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT GCT CAG CGA TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAT TCC 48 Met Gly Ser Gln Val Ser Ala Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAA GAC TCTTAT GCT GCA ACA GCAGGC AAA CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCT GAT AAG TTT GCTAAC CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCT GCG CCA CTG AAG207Ala AlaPro Leu Lys 65 [0055] array number: -- die-length [of 13 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- enterovirus type 71 share name: -- notation:CDS existence location: showing the description description of 0872/89 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr ThrThr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TATGCT GCC ACA GCA GGCAAA CAG AGC CTT AAA CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCAAAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys65 [0056] array number: -- die-length [of 14 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 2587/89 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAT GAA AAC TCT 48

Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG
 GGT TCC ACC ATA AAC TAC ACT ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile
 Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCCTAT GCC GCC ACA GCAGGC AAA
 CAG AGC CTTAAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35
 40 45 GAT CCA GAT AAG TTT GCAAAT CCT GTC AAG GAT ATT TTC ACT GAA ATG 192
 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA
 AAG207Ala Ala Pro Leu Lys 65 [0057] array number: -- die-length [of 15 arrays]: -- mold [of 207
 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like
 array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: --
 notation:CDS existence location: showing the description description of 2603/89 array -- the 1-207th
 base sequences of the gene field which carries out the code of the ****4 protein of information
 enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA
 CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser
 Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA
 AAC TAT ACC ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn
 Tyr 20 25 30 TAC AAG GAC TCC TAT GCT GCC ACA GCA GGC AAA CAG AGC CTT AAA
 CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA
 GAT AAG TTT GSA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys
 Phe Xaa Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG207Ala
 AlaPro Leu Lys 65 [0058] array number: -- die-length [of 16 arrays]: -- mold [of 207 arrays]: --
 number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: --
 cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the
 description description of 0445/90 array The notation:CDS existence location to express : 1 -207
 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT GCT CAG CGA TCC
 GGC TCC of the gene field which carries out the code of the ****4 determined protein of information
 enterovirus 71 mold besides approach:E CAC GAG AAC TCC 48 Met Gly Ser Gln Val Ser Ala Gln
 Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC
 ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25
 30 TAC AAA GAC TCTTAT GCT GCA ACA GCAGGC AAA CAG AGC CTCAAA CAA 144 Tyr
 Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCT GAT AAG TTT
 GCTAAC CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val
 Lys Asp Ile Phe Thr Glu Met 50 55 60 GCT GCG CCA CTG AAG207Ala AlaPro Leu Lys 65 [0059]
 array number: -- die-length [of 17 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid
 chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA
 origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location:
 showing the description description of 0375/90 array -- the 1-207th base sequences of the gene field
 which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E
 which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC
 GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96
 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT
 GCT GCC ACA GCA GGC AAA CAG AGC CTT AAA CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr
 Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCA AAT CCT GTC AAA
 GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu
 Met 50 55 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys65 [0060] array number: -- die-
 length [of 18 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand
 topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: --
 71 shares of enterovirus type name: -- the description description of 2136/90 array The notation:CDS
 existence location to express : 1 -207 description The 1-207th base sequence array ATG GGC TCA
 CAG GTG TCC ACA CAA CGC TCC GGC TCA of the gene field which carries out the code of the
 ****4 determined protein of information enterovirus 71 mold besides approach:E CAT GAA AAC TCT

48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCC ACT
GAG GGC TCC ACC ATA AAC TAC ACT ACT ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser
Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCCTAT GCC GCT ACA GCAGGC
AAA CAG AGC CTCAAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys
Gln 35 40 45 GAT CCA GAT AAG TTT GCAAAT CCT GTC AAA GAT ATT TTC ACT GAA ATG
192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA
CTA AAG 207 Ala Ala Pro Leu Lys 65 [0061] array number: -- die-length [of 19 arrays]: -- mold [of
207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-
like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -
- the description description of 2398/90 array The notation:CDS existence location to express : 1 -207
description The 1-207th base sequence array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC
GGC TCA of the gene field which carries out the code of the ****4 determined protein of information
enterovirus 71 mold besides approach:E CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg
Ser Gly Ser His Glu Asn Ser 10 AAC TCA GCT ACT GAG GGC TCC ACC ATA AAC TAC ACT
ACT ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 30 TAC
AAG GAC TCC TAT GCC-GCT-ACA-GCA-GGC AAA CAG AGC CTC AAG CAG 144 Tyr Lys Asp
Ser Tyr Ala Ala-Thr-Ala-Gly-Lys-Gln-Ser-Leu-Lys-Gln 40 GAT CCA GAT AAG TTT GCA AAT
CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp
Ile Phe Thr Glu Met 50 60 GCA GCG CCA CTA AAG 207 Ala Ala Pro Leu Lys 65 [0062] array
number: -- die-length [of 20 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: --
single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living
thing name: -- enterovirus type 71-share name: -- the description description of 4094/90 array The
notation:CDS existence location to express : 1 -207 description The 1-207th base sequence arrays ATG
of the gene field which carries out the code of the ****4 determined protein of information enterovirus
71 mold besides approach:E GGC TCA CAG GTG TCC ACA CAG CGC TCC GGC TCG CAT GAA
AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA
GCT ACC GAG GGC TCC ACC ATA AAC TAC ACT ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu
Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT GCC GCT ACA
GCA GGC AAA CAGAGT CTT AAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln
Ser Leu Lys Gln 35 40 45 GAT CCA GAC AAG TTT GCA AAT CCT GTC AAA GAT ATTTTC
ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60
GCA GCG CCA CTA AAA 207 Ala Ala Pro Leu Lys 65 [0063] array number: -- die-length [of 21
arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class
[of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of
enterovirus type name: -- the description description of 0419/90 array The notation:CDS existence
location to express : 1 -207 description The 1-207th base sequence array ATG GGC TCA CAG GTG
TCC ACG CAA CGC TCC GGC TCG of the gene field which carries out the code of the ****4
determined protein of information enterovirus 71 mold besides approach:E CAT GAA AAC TCT 48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACT GAG
GGC TCC ACC ATA AAC TAT ACC ACC ATT AAT TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile
Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCCTAT GCC GCC ACA GCAGGC AAA
CAG AGT CTTAAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35
40 45 GAT CCA GAC AAG TTT GCAAAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCT GCG CCA CTA
AAG 207 Ala Ala Pro Leu Lys 65 [0064] array number: -- die-length [of 22 arrays]: -- mold [of 207
arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like
array]: -- cDNA to genomic RNA origin living thing name: -- 16 shares of enterovirus coxsackievirus A
name: -- the description description of G10 array The notation:CDS existence location to express : 1 -
207 description The 1-207th base sequence array ATG GGG TCA CAA GTC TCA ACC CAA CGA
TCG GGT of the gene field which carries out the code of the ****4 determined protein of information
Coxsackie A group virus 16 mold besides approach:E TCC CAC GAA AAT TCG 48 Met Gly Ser Gln

Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCA TCA GAA GGA TCT ACT
ATA AAC TAC ACC ACC ATC AAC TAT 96 Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile
Asn Tyr 20 25 30 TAC AAG GAT GCA TAT GCT GCC AGC GCG GGT CGC CAA GAT ATG TCT
CAG 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln 35 40 45 GAC CCTAAG
AAA TTC ACA GAC CCT GTG ATG GAT GTC ATA CAC GAG ATG 192 Asp Pro Lys Lys Phe Thr
Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCT CCC TTG AAA 207 Ala Pro Pro Leu
Lys65 [0065] array number: -- die-length [of 23 arrays]: -- mold [of 207 arrays]: -- number [of
nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to
genomic RNA origin living thing name: -- 16 shares of coxsackievirus A name: -- the description
description of 1547/79 array The notation:CDS existence location to express : 1 -207 description The 1-
207th base sequence array ATG GGG TCA CAG GTT TCC ACT CAG CGG TCT GGG of the gene
field which carries out the code of the ****4 determined protein of information Coxsackie A group virus
16 mold besides approach:E TCA CAT GAG AAC TCA48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser
Gly Ser His Glu Asn Ser 5 10 15 AAC TCT GCA TCG GAG GGT TCA ACT ATA AAT TAT ACA
ACC ATA AAT TAC 96 Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30
TAT AAG GAT GCA TAT GCT GCA AGT GCGGGG CGC CAG GAT ATGTCC CAA 144 Tyr Lys
Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln 35 40 45 GAC CCG AAG AAA TTT
ACCGAT CCT GTT ATG GAC GTT ATA CAT GAG ATG 192 Asp Pro Lys Lys Phe Thr Asp Pro
Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCA CCA CTT AAA 207 Ala Pro Pro Leu Lys65
[0066] array number: -- die-length [of 24 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid
chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA
origin living thing name: -- 16 shares of coxsackievirus A name: -- notation:CDS existence location:
showing the description description of 4057/81 array -- the 1-207th base sequences of the gene field
which carries out the code of the ****4 protein of information Coxsackie A group virus 16 mold besides
approach:E which determined 1 -207 description Array ATG GGG TCA CAG GTC TCC ACT CAG
CGG TCT GGG TCA CAT GAG AAC TCA 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His
Glu Asn Ser 5 10 15 AAC TCT GCA TCG GAG GGT TCA ACT ATA AAT TAC ACA ACC ATA
AAT TAC 96 Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAT AAG
GAT GCA TAT GCT GCA AGT GCG GGG CGC CAG GAT ATG TCC CAA 144 Tyr Lys Asp Ala
Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln 35 40 45 GAC CCG AAG AAA TTT ACC GAT
CCT GTC ATG GAC GTT ATA CAT GAG ATG 192 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp
Val Ile His Glu Met 50 55 60 GCT CCA CCA CTC AAA 207 Ala Pro Pro Leu Lys65 [0067] array
number: -- die-length [of 25 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: --
single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living
thing name: -- 16 shares of coxsackievirus A name: -- the description description of 0216/86 array The
notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG
GGG TCA CAG GTC TCC ACT CAG SGG TCT GGG of the gene field which carries out the code of
the ****4 determined protein of information Coxsackie A group virus 16 mold besides approach:E TCA
CAC GAA AAC TCA48 Met Gly Ser Gln Val Ser Thr Gln Xaa Ser Gly Ser His Glu Asn Ser 5 10 15
AAC TCT GYA TCG glass fiber reinforced gypsum GGT ACA WCT ATA AAT TAC ACA CCC
ATA AAT TAC 96 Asn Ser Xaa Ser Xaa Gly Thr Xaa Ile Asn Tyr Thr Pro Ile Asn Tyr 20 25 30 TAT
AAG GAT GCA TAT GCT GCA AGT GCG GGACGM CAG GAT ATGTCC CAG 144 Tyr Lys Asp
Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln 35 40 45 GAC CCG AAG AAA TTC ACCGAT
CCT GTC ATG GAC GTT ATA CAT GAG ATG 192 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp
Val Ile His Glu Met 50 55 60 GCT CCA CCG CTC AAA 207 Ala Pro Pro Leu Lys65 [0068] array
number: -- die-length [of 26 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: --
single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living
thing name: -- 16 shares of coxsackievirus A name: -- notation:CDS existence location: showing the
description description of 0241/91 array -- the 1-207th base sequences of the gene field which carries
out the code of the ****4 protein of information Coxsackie A group virus 16 mold besides approach:E
which determined 1 -207 description Array ATG GGG TCA CAG GTC TCC ACT CAA CGG TCT

GGG TCA CAT GAG AAC TCA 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn
 Ser 5 10 15 AAC TCA GCA TCA GAG GGT TCA ACT ATA AAT TAC ACA ACC ATA AAT TAC
 96 Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAT AAA GAT GCA
 TAT GCT GCG AGT GCG GGG CGC CAG GAT ATG TCC CAA 144 Tyr Lys Asp Ala Tyr Ala Ala
 Ser Ala Gly Arg Gln Asp Met Ser Gln 35 40 45 GAT CCG AAG AAA TTT ACC GAT CCT GTT ATG
 GAT GTT ATA CAC GAG ATG 192 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu
 Met 50 55 60 GCT CCA CCA CTC AAA 207Ala Pro Pro LeuLys 65 [0069] array number: -- die-length
 [of 27 arrays]: -- mold [of 16 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: --
 nucleic acid besides class: of a straight chain-like array A part of information enterovirus of
 5'untranslation region besides a synthetic DNA Array array CTACTTTGGG TGTCCG which has a
 complementarity in antigenomic sense RNA 16 [0070] array number: -- die-length [of 28 arrays]: --
 mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid
 besides class: of a straight chain-like array A part of gene field which carries out the code of the ****2
 protein of information enterovirus besides a synthetic DNA Array which has a complementarity in
 genomic sense RNA. Q of the 6th base number shows T or C during an array, and N of the 18th base
 number shows A, C, G, or T.

Array GGTAQTTC ACCACCANCC 20 [0071] array number: -- die-length [of 29 arrays]: -- mold
 [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides
 class: of a straight chain-like array A part of information enterovirus of 5'untranslation region besides a
 synthetic DNA Array array CATCTTTGGG TGTCCGTGTT which has a complementarity in
 antigenomic sense RNA 20 [0072] array number: -- die-length [of 30 arrays]: -- mold [of 20 arrays]: -
 - number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight
 chain-like array A part of gene field which carries out the code of the VP2 protein of information
 enterovirus besides a synthetic DNA genomic sense Array array TCAGGCAACT TCCACCACCA
 which has a complementarity in RNA 20 [0073] array number: -- die-length [of 31 arrays]: -- mold [of
 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class:
 of a straight chain-like array The 118-137th of the gene field which carries out the code of the ****4
 protein of information enterovirus besides a synthetic DNA Array array AGGCTCTGTT
 TGCCTGCTGT which has a complementarity in antigenomic sense RNA 20 [0074] array number: --
 die-length [of 32 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand
 topology: -- nucleic acid besides class: of a straight chain-like array The 118-137th of the gene field
 which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA
 Array array ATATCCTGGC GCCCGCACT which has a complementarity in antigenomic sense RNA
 20 [0075] array number: -- die-length [of 33 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-
 acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The
 122-141st of the gene field which carries out the code of the ****4 protein of information enterovirus
 besides a synthetic DNA Array array TTTGAGGCTC TGTTTGCTG which has a complementarity in
 antigenomic sense RNA 20 [0076] array number: -- die-length [of 34 arrays]: -- mold [of 20 arrays]: -
 - number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight
 chain-like array The 122-141st of the gene field which carries out the code of the ****4 protein of
 information enterovirus besides a synthetic DNA Array array GGACATATCC TGGCGCCCCG which
 has a complementarity in antigenomic sense RNA 20 [0077] array number: -- die-length [of 35 arrays]:
 -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid
 besides class: of a straight chain-like array The 181-200th of the gene field which carries out the code of
 the ****4 protein of information enterovirus besides a synthetic DNA Array array GGCGCTGCCA
 TTTCAAGTGA which has a complementarity in antigenomic sense RNA 20 [0078] array number: --
 die-length [of 36 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand
 topology: -- nucleic acid besides class: of a straight chain-like array The 181-200th of the gene which
 carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array
 array GGTGGAGCCA TCTCATGTAT which has a complementarity in antigenomic sense RNA 20
 [0079] array number: -- die-length [of 37 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid

chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 173-192nd of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array which has a complementarity in antigenomic sense RNA. Q of the 19th base number shows C or T during an array.

Array CATTTTCAGTG AAAATRTCQT 20 [0080] array number: -- die-length [of 38 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 173-192nd of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array CATCTCRTGT

ATAACRTCCA which has a complementarity in antigenomic sense RNA 20 [0081] array number: -- die-length [of 39 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 118-137th of the gene field which carries out the code of ****4 of information enterovirus besides a synthetic DNA Array array ATATCTTGAC GCCCAGCGCT which has the complementarity of antigenomic sense RNA 20 [0082] array number: -- die-length [of 40 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 172-191st of the gene field which carries out the code of ****4 of information enterovirus besides a synthetic DNA Array which has the complementarity of antigenomic sense RNA. Q of the 3rd base number shows C or T during an array.

Array ATQTCATGTA TAACRTCCAT 20 [0083] array number: -- die-length [of 41 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 172-191st of the gene field which carries out the code of ****4 of information enterovirus besides a synthetic DNA Array which has the complementarity of antigenomic sense RNA. Q of the 18th base number shows C or T during an array.

Array ATTTTCAGTGA AAATRTCQTT 20

[Translation done.]

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TECHNICAL FIELD

[The technical field to which invention belongs] This invention relates to the base sequence in the differentiation approach of the enterovirus 71 mold (this may be written as "EV71" below) belonging to enterovirus, and Coxsackie A group virus 16 mold (this may be written as "CA16" below), the DNA probe used for it, and the gene field which carries out the code of the ****4 protein of enterovirus 71 mold and Coxsackie A group virus 16 mold to a list.

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PRIOR ART

[Description of the Prior Art] In order to classify the enterovirus (Enterovirus) belonging to the Picornaviridae (Picornaviridae) into about 70 kinds of human serum protein types and to show a variegated infectious disease, it is difficult to presume the virus which becomes a cause from a clinical manifestation. Therefore, separation identification of a virus is needed for deciding a pathogen. However, a current enterovirus separation method of identification separates a virus using cultivation, and the protection test is still more nearly required for it for identification. And two - four weeks is required for the isolation culture of these viruses. Furthermore, in the protection test which used the neutralization antiserum of a standard stock, the separation stock in which human serum protein type differentiation is impossible appears frequently. This is considered for the gene of enterovirus to vary extremely in a nature at high speed, and production of the antiserum which always neutralizes a fresh separation stock is needed for these solutions.

[0003] High sensitivity and polymerase chain reaction method [Polymerase Chain Reaction which amplifies DNA specifically Law, ; which writes this as the "PCR method" below -- Saiki and others, Science, 230 volumes, p.1350-1354, and 1985 After year reference] is developed, enterovirus The PCR method using a primer complementary to the base sequence of 5'untranslation region, 5' -- in an untranslation region ****4 and ****2 protein To the base sequence of the gene field which carries out a code, a complementarity The primer which it has [Rotbart and H. which are detected by the used PCR method, 5.J.Clinical Microbiology, 28, and 438-442 (1990); Olive and D.M. and 5 J.General Virology, 71, 2141-2147(1990)].

[0004] Furthermore, mold identification of the enterovirus by the stringent reverse solid phase hybridization method using DNA amplified by PCR is reported [clinical [, such as **** Hiroaki,], a virus, 22 volumes, and page 199-207 (1994)]. However, in this experiment, the reactivity of a target gene and a probe changes with differences in an age or an area also in the same human serum protein type, and it is expected to be difficult to select a specific viral strain to a probe. In the disease by enterovirus, since the virus separated from the patient occupies 90% or more by EV71 and CA16, hand, foot and mouth disease can identify the epidemic virus of hand, foot and mouth disease by detecting quickly and simply two sorts of human serum protein types.

[Translation done.]

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] This invention aims at offer of the approach of carrying out mold differentiation of EV71 and CA16 in a high precision.

[Translation done.]

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MEANS

[Means for Solving the Problem] As a result of repeating examination wholeheartedly that the above-mentioned purpose should be attained, in the base sequence of the gene field which carries out the code of the ****4 protein of EV71 and CA16, this invention persons had a specific base sequence in each human serum protein type, find out that EV71 and CA16 can discriminate in a high precision using this base sequence, and came to complete this invention. That is, this invention provides following 1-6 with the DNA probe and DNA fragment which are used for the mold differentiation approach of the enterovirus 71 mold of a publication, and Cocksackie A group virus 16 mold, and it.

[0007] 1. (i) enterovirus 71 mold and Cocksackie A group virus 16 mold, The DNA field which carries out the code of some ****4 and ****2 proteins which have a specific DNA array in a part of 5'untranslation region and the human serum protein type of enterovirus is amplified. (ii) The DNA array of the DNA field which carries out the code of the ****4 protein under this magnification DNA is determined. It is based on a DNA array specific to each human serum protein type of the enterovirus 71 mold under this DNA array, and Cocksackie A group virus 16 mold. (iii) Design the DNA array which has a complementarity respectively in this DNA array, respectively, and it is made into a DNA probe. (iv) The mold differentiation approach of the enterovirus 71 mold characterized by analyzing a binding affinity with the magnification DNA obtained by the approach of this DNA probe and the above (i), and Cocksackie A group virus 16 mold.

[0008] 2. DNA Array Which Has DNA Field Which Carries Out Code of the ****4 Protein of Enterovirus 71 Mold under Magnification DNA, and Complementarity It is the DNA array shown by the array number 31, the array number 33, the array number 35, the array number 37, or the array number 41. The approach of one above-mentioned publication that the DNA array which has the gene field which carries out the code of the ****4 protein of the Cocksackie A group virus 16 mold under magnification DNA, and a complementarity is a DNA array shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, or the array number 40.

3. DNA probe which has magnification DNA including DNA array shown by array number 31, array number 33, array number 35, array number 37, or array number 41 of DNA field which carries out code of ****4 protein of enterovirus 71 mold, and complementarity.

[0009] 4. DNA probe which has magnification DNA including DNA array shown by array number 32, array number 34, array number 36, array number 38, array number 39, or array number 40 of DNA field which carries out code of ****4 protein of Cocksackie A group virus 16 mold, and complementarity.

5. DNA fragment which is ****4 protein of enterovirus 71 mold and carries out code of protein including amino acid sequence shown by array number 1 in the amino acid sequence.

6. DNA fragment which is ****4 protein of Cocksackie A group virus 16 mold, and carries out code of protein including amino acid sequence shown by array number 2 in the amino acid sequence. Hereafter, it explains for details further about this invention.

[0010] Especially in operation of this invention, unless it is directed, the conventional technique in the molecular biology in technical within the limits of the field concerned, microbiology, a recombinant DNA, and immunology is adopted. Such technique is explained in detail into reference. For example,

please refer to the following reference. Maniatis, Fitch, and separate volume (1991); volume for University of Tokyo Institute of Medical Science carcinostatic research sections, and cell technology experiment protocol . Sambrook, MOLECULAR CLONING (1991); A LABORATORY MANUAL (1982); Kevin struhl and others, CURRENT PROTOCOLS IN MORECULAR BIOLOGY, one volume, and two volumes; the volume for Masami Muramatsu, lab manual gene engineering (1990) ; Masami Muramatsu The volume for Hiroto Okayama, a gene engineering handbook, experimental medicine [0011] 1. Enterovirus 71 Mold And a part of 5'untranslation region of Cocksackie A group virus 16 mold A specific base sequence to the human serum protein type of enterovirus Some ****4 and ****2 proteins which it has a code -- carrying out -- a gene -- a field -- magnification -- enterovirus -- 71 -- a mold -- and -- Cocksackie -- A -- a group -- a virus -- 16 -- a mold -- five -- ' -- an untranslation region -- a part -- enterovirus -- a human serum protein type -- being specific -- a base sequence -- having -- **** -- four -- and -- **** -- two -- protein -- a part -- a code -- carrying out -- a gene -- a field (below this) Magnification which may be called for short "a gene field including the human serum protein type specific base sequence of enterovirus" can be performed as follows.

[0012] First, the enterovirus isolation culture stock of known [human serum protein type] and the human serum protein type by which subculture is carried out extract RNA from a known enterovirus standard stock etc. with a conventional method, and produces cDNA from this extract RNA using reverse transcriptase. The die length which includes the gene field which carries out the code of 5'untranslation region of enterovirus 71 mold and Cocksackie A group virus 16 mold, and ****4 and ****2 for the oligonucleotide which has a complementarity as a primer in the mold intersection of the upstream of the gene field which includes the human serum protein type specific base sequence of enterovirus in this cDNA, and a down-stream mold intersection amplifies the gene DNA field of about 650 bases.

[0013] the PCR method for which magnification of a gene is usually used -- [-- JP,61-274697,A, JP,62-281,A, 239 Sakai sScience(s), and p.487-491 reference] can perform the detail of this PCR method easily. As long as it uses for coincidence the oligonucleotide which has a complementarity in the mold intersection of the upstream of a gene field including a human serum protein type specific base sequence, and a down-stream mold intersection as an oligonucleotide (this may be called for short a "human serum protein type common primer" below) which can be used as a primer on the occasion of magnification of a gene field including the human serum protein type specific base sequence of enterovirus, you may be what kind of oligonucleotide. It is appropriate to use as a primer the oligonucleotide which was specific to enterovirus, and set the high base sequence of similarity as 5'untranslation region (upstream mold intersection) and ****2 field (down-stream mold intersection) between seeds, and carried out chemosynthesis in them based on the base sequence based on desirable known human serum protein type specific base sequence data.

[0014] As an example of the above-mentioned primer, they are D.M.Olive et al, 71 Journal of General Virology, and a page. 2141-2147 (1990) The oligonucleotide shown by the array number 29 and the array number 30 which are indicated by the oligonucleotide shown by the array number 27 and the array number 28 which are indicated, and the JP,6-311900,A specification which invention-in-this-application persons proposed previously can be mentioned. the inside of these primers -- 5' -- the oligonucleotide shown by the array number 29 as a primer by the side of an end, and 3' -- it is appropriate to use for coincidence the oligonucleotide shown by the array number 28 as a primer by the side of an end.

[0015] Chemosynthesis of the above-mentioned primer can be easily carried out in itself with the solid phase synthesis method using a model 381-ADNA composition machine, known the nucleic-acid-biosynthesis equipment usually used, for example, applied biotechnology systems (Applied Biosystems) company make, etc. It can dissociate with a conventional method, for example, agarose gel electrophoresis, and the above-mentioned magnification DNA can be detected as a DNA band, and, thereby, can check the gene DNA of the enterovirus 71 mold and Cocksackie A group virus 16 mold origin.

[0016] 2. The base sequence of the decision above-mentioned magnification DNA of the base sequence of the gene field which carries out the code of the ****4 protein under magnification DNA is for

example, applied biotechnology systems company make and model 373-A. An auto sequencer is used and it is Dye DeoxyTM. It can determine by the terminator method. The base sequence of the gene field which carries out the code of the ****4 protein can be determined by searching for the base sequence [a protein nucleic-acid enzyme, 137 volumes, No. 14, and page 2609-2618 (1992) reference] corresponding to the amino acid sequence which cuts ****4 known protein and ****2 known protein of the translation initiation codon in the determined base sequence, and enterovirus.

[0017] The array of the gene field which carries out the code of the ****4 protein of EV71 obtained in this way is shown in the array number 3 - the array number 21, and the combination of the amino acid sequence is shown in the array number 1. Moreover, the array of the gene field which carries out the code of the ****4 protein of CA16 is shown in the array number 22 - the array number 26, and the combination of the amino acid sequence is shown in the array number 2. The DNA fragment of this invention which includes the above-mentioned base sequence is the DNA synthesizer usually used only not only in cDNA compounded from RNA of EV71 and CA16 separation stock, for example, the above-mentioned applied biotechnology systems company make, and model 381-A. It may be compounded using a DNA synthesis machine.

[0018] 3. The base sequence of the DNA probe (this may be called a "EV71 unique probe" for short below) which has a complementarity in the specific base sequence of the design and synthetic enterovirus 71 mold of a DNA probe can be designed by analyzing a specific array to EV71 under array shown by said array number 3 - the array number 21. Similarly, the base sequence of the DNA probe (this may be called a "CA16 unique probe" for short below) which has a complementarity in the specific base sequence of Coxsackie A group virus 16 mold can be designed by analyzing a specific array to CA16 under array shown by said array number 22 - the array number 26.

[0019] As an EV71 unique probe obtained in this way, the oligonucleotide shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, or the array number 40 can be mentioned, for example, for example as the oligonucleotide shown by the array number 31, the array number 33, the array number 35, the array number 37, or the array number 41, and a CA16 unique probe. The oligonucleotide shown as an EV71 unique probe by the array number 32, the array number 39, and the array number 40 as the array number 31 and the array number 41, and a CA16 unique probe can be preferably mentioned among the above-mentioned human serum protein type unique probes. The above-mentioned DNA probe is model 381-A, known the nucleic-acid-biosynthesis equipment usually used, for example, applied biotechnology systems company make, in itself. With the solid phase synthesis method using a DNA synthesis machine etc., chemosynthesis can be carried out easily.

[0020] 4. EV71 and CA16 can be judged using a human serum protein type [in which the enterovirus 71 mold and the coxsackie virus 16 mold carried out the mold differentiation above] unique probe as follows. First, viral RNA is extracted from the virus isolation culture stock from the clinical specimen extracted at the time of a medical examination with a conventional method. The gene field which carries out the code of some 5'untranslation region [of enterovirus / a part of], ****4, and ****2 proteins after compounding cDNA using said human serum protein type common primer from this extract RNA is amplified by said PCR method. Detection of Magnification DNA can separate Magnification DNA by agarose electrophoresis, can be made to be able to color it by UV irradiation etc. after dyeing with a suitable stain, for example, the ethidium bromide etc., and can be performed by checking the DNA band.

[0021] For the DNA fragment amplified using said human serum protein type common primer, and detection of said human serum protein type unique DNA probe 71, i.e., EV, mold differentiation of EV71 and CA16 The array shown by the array number 31, the array number 33, the array number 35, the array number 37, and the array number 41 again for example, for detection of CA16 For example, the DNA probe produced by carrying out the indicator of the oligonucleotide which has the array shown by the array number 32, the array number 34, the array number 36, the array number 38, the array number 39, and the array number 40 It can be made to be able to hybridize with a conventional method on solid phase, and the class of human serum protein type unique DNA probe can be performed by

carrying out detection analysis.

[0022] Although association of DNA to a solid phase top can be performed by carrying out the chemical bond of the approach DNA, for example, the magnification, usually using known in itself with a conventional method to suitable solid phase, for example, a nylon membrane, after denaturalizing The approach (this may be called the "Southern-blotting method" for short below) of neutralizing the above-mentioned agarose gel preferably used in order to check Magnification DNA after denaturalizing, for example, fixing to a nylon membrane after transferring Magnification DNA is mentioned.

[0023] The phosphorylation of 5'end can perform the indicator of a DNA probe using [gamma-32P] ATP and T-four polynucleotide kinase (Toyobo Co., Ltd. make). Moreover, detection of an indicator object can perform the autoradiography of the nylon membrane which performed hybridization with the DNA probe by which the indicator was carried out, and can be performed by investigating the existence of hybridization with each DNA probe.

[0024] Examples, such as a DNA fragment which can be amplified, are shown in drawing 17 using the field which carries out the code of some a part of gene field including above-mentioned human serum protein type specific DNA array of enterovirus used by this invention, i.e., 5'untranslation region of enterovirus, ****4, and ****2 proteins, a human serum protein type common primer, a DNA probe, and this primer. As for the location (base pair number) of the gene field where double digits carry out the code of an array number and the ****4 protein with which a DNA probe combines the figure in (), the magnitude of the DNA fragment with which the figure in [] is amplified, and bp, a base pair is shown among drawing.

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EXAMPLE

[Example] Next, although an example is given and this invention is further explained to a detail, the following example is given only as an aid which acquires recognition concrete about this invention, and the range of this invention is not limited at all by this.

[0026] Example 1 It experimented using the enterovirus separation stock and standard stock with which it dissociated from the patient of (Sequence A) use microorganism following of EV71 and CA16 isolation-culture stock, and the human serum protein type was identified by the protection test using the specific antiserum. The standard stock used here is a virus standard stock by which subculture is carried out in the National Institute of Health.

[0027]

(1) Enterovirus separation stock Stock name (human serum protein type) Separation stage Coxsackie A group virus 16 mold (CA16)

1547/79 1979 4057/81 1981 0216/86 1986 0241/91 1991 Enterovirus 71 mold (EV71)

NAGOYA/70 1970 0108/78 1978 3059/78 1978 3359/83 1983 4132/85 1985 T86236a / 861986 years 1091/89 1989 year 0445 / 90 1990 0004/78 1978 year 1096 / 86 1986 0872/89 1989 year 2587 / 89 1989 2603/89 1989 year 0375 / 90 1990 2136/90 1990 year 2398 / 90 1990 4094 / 90 1990 0419/90 1990

[0028] (2) Standard **** name (human serum protein type) Separation stage Coxsackie A group virus 16 molds (CA16)

Enterovirus 71 molds (EV71)

[0029] (B) From the extract above-mentioned virus liquid of RNA, the SUMAI test R kit (Sumitomo Metal, Ltd. make) extracted RNA, and isopropanol precipitation was performed.

(C) cDNA which originates in each virus using a reverse transcriptase (product made from GIBCO/BRL) by using as mold RNA obtained by the synthetic above-mentioned (B) term of cDNA was compounded.

(D) the oligonucleotide shown by the synthetic array number 28 and the array number 29 of a primer for PCR -- phospho friend DAIDO (Phosphoamidide) -- law -- applied biotechnology systems company make and model 381-A It compounded using the DNA synthesis machine. The above-mentioned oligonucleotide was refined using the OPCTM cartridge, and it was used as a primer of PCR.

[0030] (E) Target's DNA magnification (PCR method)

Reaction mixture is 10mM(s) about final concentration. Tris-HCl (pH8.0), 50mM(s) KCl, 0.001% gelatin, 0.55mM MgCl₂, 0.05mM(s) In dNTPs and the solution made into the formamide 2.5% Above-mentioned oligonucleotide composition primer (array number 28 and array number 29; every 50microM) every 0.1microl, enterovirus cDNA compounded by the aforementioned (C) term 100ng-1microg and Taq polymerase (made in Japanese Roche) 0.125microl (0.625U) -- adding -- total 50microl ** -- what was carried out was prepared. PCR is 95-degree-C 30 seconds, and 55-degree-C reaction condition for 30 seconds, and amplified Target DNA using the amplification system (Amplification System; SHITASU) in 14 cycle.

[0031] Furthermore, they are 10mM(s) about final concentration to the above-mentioned solution. Tris-HCl (pH8.0), 50mM(s) KCl, 0.001% gelatin, 2.35mM MgCl₂, 0.15mM(s) In dNTPs and the solution

made into the formamide 2.5% Above-mentioned oligonucleotide composition primer (array number 28 and array number 29; every 50microM) every 1.0microl, Total 50microl which added Taq polymerase 0.25microl (1.25U) The solution was added and it amplified in 95-degree-C 30-second and 55-degree-C 30 seconds, and 72-degree-C 45 seconds and 40 cycle.

[0032] (F) The ethidium bromide was added to agarose gel of 3.0% of checks of the magnification DNA by gel electrophoresis ml 0.5microg /, and electrophoresis of DNA amplified by the above-mentioned (E) term was performed. 254nm ultraviolet rays were irradiated after migration, the coloring reaction of the ethidium bromide detected the DNA band, and the target DNA band of about 650 bases originating in the gene field which carries out the code of a part of 5'untranslation region of enterovirus and some ****4 and ****2 proteins was detected.

[0033] (G) Centricon-100 (Amicon make) is used for the PCR product checked by the decision above (F) of the base sequence of ****4 field, and it is after purification and Dye DeoxyTM. The terminator method Cycle Sequencing It reacts and is model by applied biotechnology systems company 373-A. The base sequence was determined using the DNA sequencer. By searching for the base sequence corresponding to the amino acid sequence which cuts ****4 known protein and ****2 known protein of the translation initiation codon in the determined base sequence, and enterovirus, the base sequence of the gene field which carries out the code of the ****4 protein was determined. The array which carries out the code of the ****4 protein of CA16 for the array which carries out the code of the ****4 protein of EV71 obtained in this way to the array number 3 - the array number 21 is shown in the array number 22 - the array number 26.

[0034] Example 2 It experimented using the 39 following kinds of human serum protein type enterovirus standard stocks by which subculture is carried out in the mold (differentiation A) use microorganism (1) standard stock National Institute of Health of EV71 and CA16. Each of such enteroviruses is standard stocks with which the human serum protein type is identified by the protection test which used the specific antiserum.

[0035]

A stock name (human serum protein type) A cable address The Coxsackie A group virus 2 molds CA2 ** 4** CA4 ** 6** CA6 ** 7** CA7 ** 9** CA9** 10 ** CA10 ** 16 ** CA16 **24** CA24 The Coxsackie B group virus 1 mold CB1 **2** CB2 ** 3** CB3** 4** CB4 ** 5** CB5** 6** CB6 Echovirus 1 mold E1 ** 3** E3 **4** E4 ** 5** E5 ** 6** E6 ** 7** E7 ** 9** E9 ** 11 ** E11 ** 12** E12** 13** E13** 14** E14** 16** E16 ** 17** E17 ** 18** E18 ** 19 ** E19 ** 21** E21 ** 22** E22 ** 24 ** E24 ** 25** E25 ** 30 ** E30 Enterovirus 70 molds EV70 ** 71**EV71

Poliovirus 1 mold PV1 ** 2 ** PV2 ** 3 ** PV3 [0036] (2) It experimented using the enterovirus separation stock with which it dissociated from the patient of the enterovirus separation stock following, and the human serum protein type was identified by the protection test using the specific antiserum.

[0037]

Stock name Separation stage Coxsackie A group virus 16 mold (CA16)

4057/81 1981 0843/87 1987 0071/88 1988 1045/90 1990 1051/90 1990 0241/90 1990 2500/92 1992 enterovirus 71 mold (EV71)

NAGOYA/70 1970 2105/73 1973 2089/73 1973 2146/73 1973 2203/73 1973 2377/73 1973 2381/73 In 1973 0108/78 1978 3059/78 In 1978 0193/78 1978 0297/78 In 1978 0333/78 1978 0761/82 1982 year FE-27 1982 0272/83 1983 year FE-18 1983 FE-20 1983 4132/85 1985 0866/86 1986 0891/86 In 1986 0948/86 1986 1025/86 In 1986 1027/86 1986 1079/86 1986 1096/86 1986 1163/86 1986 0460/87 1987 0443/88 1988 2587/89 1989 2603 / 891989 years 2136/90 1990 2234/90 1990 2398/90 1990 0419/90 1990 0445/90 1990 0189 / 901990 years 4094/90 1990 FE-45 1990 year FE-46 1990 T91-Y563 1991 1264/92 1992 2326 / 921992 years 2909/93 1993 0089/93 1993 0133/93 1993 0136/93 1993 0146/93 1993 2200/93 1993 year 0154 / 93 1993 0159/93 1993 2122/93 1993 2167/93 1993 2168/93 1993 2173/93 1993 0565/93 1993 0630/93 In 1993 0679/93 1993 0702/93 In 1993 0724/93 1993 0748/93 In 1993 0774/93 1993 0791/93 1993 year FE-57 1993 T93-128 1993 year T93-133 1993 T93-Y998 1993

[0038] (B) As (B) - (F) term of an RNA extract, cDNA composition and magnification by PCR of an enterovirus gene, and its detection example 1 showed, RNA of the above-mentioned enterovirus

standard stock and a separation stock was extracted, and magnification was checked in the electrophoresis using agarose gel after amplifying the part corresponding to a part of 5'untranslation region and some ****4 and ****2 proteins. In addition, it is HaeIII of phix174 phage as a marker. The decomposition product was migrated to coincidence.

[0039] (C) The agarose gel used by the (B) term of the Southern blot technique example 2 for the check of Magnification DNA was put in for 30 minutes for 30 minutes with alkali denaturation liquid (0.5M NaOH, 1.5M NaCl) after processing and with neutralization liquid (1 M Tris-HCl, 1.5M NaCl), gel was returned to neutrality, and DNA was denatured. Furthermore, DNA which performed Southern blotting overnight and shifted to the nylon membrane with the conventional method was made to fix by 254nm 1.2x105micro JOULES/cm2 (UV stratalinkerTM; Stratagene make) of ultraviolet rays.

[0040] (D) The base sequence [the array number 26 from the array number 3] of the DNA field which carries out the code of the ****4 protein of CA16 and EV71 shown by a design and the synthetic example 1 (G) term of a human serum protein type specific probe was analyzed, the array number 31 and the array number 41, and the CA16 unique probe were designed like the array number 32, the array number 39, and the array number 40, and the EV71 unique-from these arrays probe compounded according to the example 1 (D) term. The phosphorylation of 5'end performed the indicator of the above-mentioned DNA probe using [gamma-32P] ATP and T-four polynucleotide kinase (Toyobo Co., Ltd. make).

[0041] (E) The membrane produced by the hybridization above-mentioned example 2 (C) term is 1.0M NaCl and 50mM as reaction mixture. Tris-HCl (pH7.5), 0.5%PVP, 0.2% heparin, 1mM It processed at 50 degrees C for 1 hour using EDTA and 2%SDS, and pre hybridization was performed. Hybridization added the [gamma-32P] ATP indicator DNA probe compounded by the (D) term of an example 2 105 cpm/ml to the above-mentioned hybridization solution, and made it react to it 50 degrees C for 4 hours. It washed at 50 degrees C in the 5xSSC-0.1%SDS solution, the membrane was washed twice at 50 degrees C in the 2xSSC-0.1%SDS solution after 5 minutes and 2 times washing for 5 minutes, and the probe combined nonspecific was removed. After drying a nylon membrane, on the lap, after the room temperature performed a package and autoradiography for 15 minutes further for 45 minutes at -80 degrees C, they were developed.

[0042] Formation of the hybrid of the CA16 specific DNA probe (the array number 32, the array number 38, and array number 39) and Magnification DNA which were obtained in this way is shown in drawing 1 - drawing 8, and the hybridization of an EV71 specific DNA probe (the array number 31 and array number 41) and Magnification DNA is shown in drawing 9 - drawing 16. The passage clear from drawing, all EV71 separation stocks react to an EV71 specific DNA probe, and all CA16 separation stocks react to a CA16 specific DNA probe, and there is no cross reaction between a human serum protein type specific DNA probe and DNA obtained from the enterovirus standard stock of each human serum protein type, and it became clear that this was specific association. According to the approach of this invention the above-mentioned passage, CA16 and EV71 can discriminate with high precision.

[0043]

[Layout Table]

array number: -- die-length [of one array]: -- mold [of 69 arrays]: -- amino acid topology: -- Ggg of under the information following array of straight chain-like others, and the 3rd amino acid number Ser Or Thr being shown -- Hhh of the 7th amino acid number Ala Or Thr It is shown and is the 9th amino acid number. Iii Arg or -- Xaa -- being shown -- Jjj of the 17th amino acid number Asn or -- Xaa -- being shown -- Kkk of the 31st amino acid number Asn Or Xaa being shown -- Lll of the 34th amino acid number Lys Or Xaa being shown -- Mmm of the 35th amino acid number Asp Or Xaa being shown -- Nnn of the 43rd amino acid number Lys Or Xaa being shown -- Ooo of the 47th amino acid number Lys or Xaa being shown -- Ppp of the 49th amino acid number Asp or Xaa It is shown and is Qqq of the 53rd amino acid number. Phe or Xaa It is shown and is Rrr of the 54th amino acid number. Ala Or Gly Or Xaa It is shown and is Sss of the 55th amino acid number. Asn Or Xaa It is shown.

Array Met Gly Ggg Gln Val Ser Hhh Gln Iii Ser Gly Ser His Glu Asn Ser 5 10 15 Jjj Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Kkk Tyr 20 25 30 TyrLll Mmm Ser Tyr Ala Ala Thr Ala Gly Nnn

Gln Ser Leu Ooo Gln 35 40 45 Ppp Pro Asp Lys Qqq Rrr Sss Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60Ala Ala Pro Leu Lys 65 [0044] array number: -- die-length [of two arrays]: -- mold [of 69 arrays]: -- amino acid topology: -- Aaa of under the information following array of straight chain-like others, and the 9th amino acid number Arg Or Xaa being shown -- Bbb of the 19th amino acid number Ala Or Xaa being shown -- Ccc of the 21st amino acid number Glu Or Xaa It is shown and is Ddd of the 23rd amino acid number. Ser Or Thr It is shown and is Eee of the 24th amino acid number. Thr Or Xaa It is shown and Fff of the 29th amino acid number is Thr. Or Pro It is shown.

Array Met Gly Ser Gln Val Ser Thr Gln Aaa Ser Gly Ser His Glu Asn Ser 5 10 15 Asn Ser Bbb Ser Ccc Gly Ddd Eee Ile Asn Tyr Thr Fff Ile Asn Tyr 20 25 30 TyrLys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln 35 40 45 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 Ala Pro ProLeu Lys 65 [0045] array number: -- die-length [of three arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus enterovirus type name: -- the description description of a BrCr array The notation:CDS existence location to express : 1 - 207 description The 1-207th base sequence array ATG GGC TCC CAG GTC TCC ACA CAG CGA TCC GGC TCG CAT GAG AAT TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 1015 AAC TCA GCC ACG GAA GGC TCC ACT ATA AAT TAC ACA ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAA GAC TCG TAT GCT GCC ACT GCT GGA AAG CAA AGT CTC AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCT GAC AAG TTT GCG AAC CCT GTG AAG GAC ATC TTT ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asplle Phe Thr Glu Met 50 55 60 GCA GCG CCC TTA AAG 207Ala AlaPro Leu Lys 65 [0046] array number: -- die-length [of four arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of NAGOYA/70 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT ACT CAG CGG TCC GGC TCC CAC GAG AAT TCC of the gene field which carries out the code of the ****4 determined protein of information en TEROUISURU71 mold besides approach:E 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 1015 AAT TCA GCT ACA GAA GGT TCC ACC ATT AAT TAC ACT ACT ATC AAT TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCA GGC AAG CAG AGC CTC AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln-Ser-Leu-Lys-Gln 35 40 45 GAC CCT GAC AAG TTT GGC-AAT-CCT-GTC-AAG GAC ATT TTC ACT GAA ATG 192Asp Pro Asp Lys Phe Gly Asn-Pro-Val-Lys-Asp-Ile-Phe-Thr-Glu-Met 50 55 60 GCG GCG CCA CTG AAG 207Ala Ala Pro Leu Lys65 [0047] array number: -- die-length [of five arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 0108/78 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT ACT CAG CGG TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAT TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATC AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCAGGT AAG CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser LeuLys Gln 35 40 45 GAC CCT GAC AAG TTT GCTAAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCC GCG CCA CTG AAG 207Ala Ala Pro Leu Lys65 [0048] array number: -- die-length [of six arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- enterovirus type

71-share name: -- the description description of 3059/73 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence arrays ATG of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E GGT TCA CAA GTG TCT ACT CAG CGG TCC GGC TCC CAC GAG AAT TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCA GGC AAG CAG AGC CTT AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCT GAC AAG TTT GCT AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCC GCG CCA CTG AAG 207Ala Ala Pro LeuLys 65 [0049] array number: -- die-length [of seven arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- enterovirus type 71 Stock name : the description description of 0004/78 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGA TCG CAG GTG TCC ACA CAA CGC TCT GGT TCG CAT GAA AAT TCT of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCC ACT GAA GGT TCC ACT ATA AAC TAC ACC ACC ATC AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAT AAG GAC TCT TAT GCC GCT ACA GCA GGC AAA CAG AGC CTTAAG CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAC AAG TTT GCA AAT CCC GTT AAA GAT ATT TTC ACT GAG ATG 192 Asp ProAsp Lys Phe Ala Asn ProVal Lys Asp Ile Phe Thr Glu Met 50 55 60 GCG GCA CCA CTG AAA 207Ala Ala Pro Leu Lys 65 [0050] array number: -- die-length [of eight arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 3359/83 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTA TCC ACT CAG CGG TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAT TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACT ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCT TAT GCT GCC ACA GCA GGC AAA CAG AGCCTC AAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu LysGln 35 40 45 GAC CCC GAC AAG TTT GCT AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Asp Pro Asp LysPhe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCG GCG CCG CTGAAG 207Ala Ala Pro Leu Lys65 [0051] array number: -- die-length [of nine arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 4132/85 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAA GTG TCT ACT CAG CGA TCC GGC TCC CAC GAG AAT TCC 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 ANT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC ANC TAT 96 Xaa Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Xaa Tyr 20 25 30 TAC ANA GNC TCT TAT GCT GCA ACA GCA GGC ANA CAG AGY CTT AMA CAA 144 Tyr Xaa Xaa Ser Tyr Ala Ala Thr Ala Gly Xaa Gln Ser Leu Xaa Gln 35 40 45 GMC CCT GAT AAG TKT GCT AMT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192 Xaa Pro Asp Lys Xaa Ala Xaa Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCC GCG CCA CTA AAA 207Ala Ala Pro Leu Lys65 [0052] array number: -- die-length [of ten arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -

- the description description of a T86236a array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT ACA CAA GTA TCC ACT CAG SGG TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAT TCT 48 Met Gly Thr Gln Val Ser Thr Gln Xaa Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAC TAC ACT ACT ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCTTAT GCT GCT ACA GCAGGC AAA CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCC GAC AAG TTT GCTAAT CCT GTC AAG GAC ATTTTT ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCG GCG CCA CTG AAG207Ala AlaPro Leu Lys 65 [0053] array number: -- die-length [of 11 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 1096/86 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGC TCC ACC ATA AAC TAT ACT ACC ATC AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT GCC GCC ACA GCA GGC AAA CAG AGC CTT AAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCG AAT CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys65 [0054] array number: -- die-length [of 12 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 1091/89 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT GCT CAG CGA TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAT TCC 48 Met Gly Ser Gln Val Ser Ala Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAA GAC TCTTAT GCT GCA ACA GCAGGC AAA CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCT GAT AAG TTT GCTAAC CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCT GCG CCA CTG AAG207Ala AlaPro Leu Lys 65 [0055] array number: -- die-length [of 13 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- enterovirus type 71 share name: -- notation:CDS existence location: showing the description description of 0872/89 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr ThrThr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TATGCT GCC ACA GCA GGCAAA CAG AGC CTT AAA CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCAAAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys65 [0056] array number: -- die-length [of 14 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description

description of 2587/89 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAC ACT ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCCTAT GCC GCC ACA GCAGGC AAA CAG AGC CTTAAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCAAAT CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG207Ala Ala Pro Leu Lys 65 [0057] array number: -- die-length [of 15 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 2603/89 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT GCT GCC ACA GCA GGC AAA CAG AGC CTT AAA CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GSA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Xaa Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys65 [0058] array number: -- die-length [of 16 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 0445/90 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGT TCA CAA GTG TCT GCT CAG CGA TCC GGC TCC of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAC GAG AAC TCC 48 Met Gly Ser Gln Val Ser Ala Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC AAC TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAA GAC TCTTAT GCT GCA ACA GCAGGC AAA CAG AGC CTCAAA CAA 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAC CCT GAT AAG TTT GCTAAC CCT GTC AAG GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCT GCG CCA CTG AAG207Ala AlaPro Leu Lys 65 [0059] array number: -- die-length [of 17 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 0375/90 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT GCT GCC ACA GCA GGC AAA CAG AGC CTT AAA CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys65 [0060] array number: -- die-length [of 18 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 2136/90 array The

notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGC TCA of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCC ACT GAG GGC TCC ACC ATA AAC TAC ACT ACT ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCCTAT GCC GCT ACA GCAGGC AAA CAG AGC CTCAAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAT AAG TTT GCAAAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys PheAla Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAG207Ala AlaPro Leu Lys 65 [0061] array number: -- die-length [of 19 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- notation:CDS existence location: showing the description description of 2398/90 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information enterovirus 71 mold besides approach:E which determined 1 -207 description Array ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGC TCA CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 10 AAC TCA GCT ACT GAG GGC TCC ACC ATA AAC TAC ACT ACT ATT AAT TAC 96 AsnSer Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 30 TAC AAG GAC TCC TAT GCC GCT ACA GCA GGC AAA CAG AGC CTC AAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 40 GAT CCA GAT AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 60 GCA GCG CCA CTA AAG 207Ala Ala Pro Leu Lys 65 [0062] array number: -- die-length [of 20 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- enterovirus type 71-share name: -- the description description of 4094/90 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence arrays ATG of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E GGC TCA CAG GTG TCC ACA CAG CGC TCC GGC TCG CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCT ACC GAG GGC TCC ACC ATA AAC TAC ACT ACC ATT AAT TAC 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAC AAG GAC TCC TAT GCC GCT ACA GCA GGC AAA CAGAGT CTT AAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln 35 40 45 GAT CCA GAC AAG TTT GCA AAT CCT GTC AAA GAT ATTTTC ACT GAA ATG 192 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met 50 55 60 GCA GCG CCA CTA AAA 207Ala Ala Pro LeuLys 65 [0063] array number: -- die-length [of 21 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 71 shares of enterovirus type name: -- the description description of 0419/90 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGC TCA CAG GTG TCC ACG CAA CGC TCC GGC TCG of the gene field which carries out the code of the ****4 determined protein of information enterovirus 71 mold besides approach:E CAT GAA AAC TCT 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAT TCA GCT ACT GAG GGC TCC ACC ATA AAC TAT ACC ACC ATT AAT TAT 96 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAC TCCTAT GCC GCC ACA GCAGGC AAA CAG AGT CTTAAG CAG 144 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln-Ser-Leu-Lys-Gln 35 40 45 GAT CCA GAC AAG TTT GCA-AAT-CCT-GTC-AAA GAT ATT TTC ACT GAA ATG 192Asp Pro Asp Lys Phe Ala Asn-Pro-Val-Lys-Asp-Ile-Phe-Thr-Glu-Met 50 55 60 GCT GCG CCA CTA AAG 207Ala Ala Pro Leu Lys 65 [0064] array number: -- die-length [of 22 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 16 shares of entrovirus coxsackievirus A name: -- the description description of G10 array The notation:CDS existence location

to express : 1 -207 description The 1-207th base sequence array ATG GGG TCA CAA GTC TCA ACC CAA CGA TCG GGT of the gene field which carries out the code of the ****4 determined protein of information Coxsackie A group virus 16 mold besides approach:E TCC CAC GAA AAT TCG 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCA TCA GAA GGA TCT ACT ATA AAC TAC ACC ACC ATC AAC TAT 96 Asn Ser Ala Ser Glu Gly Ser ThrIle Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAC AAG GAT GCA TAT GCT GCC AGC GCG GGT CGC CAA GAT ATG TCT CAG 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met SerGln 35 40 45 GAC CCTAAG AAA TTC ACA GAC CCT GTG ATG GAT GTC ATA CAC GAG ATG 192 Asp Pro Lys LysPhe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCT CCC TTG AAA 207Ala Pro Pro Leu Lys65 [0065] array number: -- die-length [of 23 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 16 shares of coxsackievirus A name: -- the description description of 1547/79 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGG TCA CAG GTT TCC ACT CAG CGG TCT GGG of the gene field which carries out the code of the ****4 determined protein of information Coxsackie A group virus 16 mold besides approach:E TCA CAT GAG AAC TCA48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCT GCA TCG GAG GGT TCA ACT ATA AAT TAT ACA ACC ATA AAT TAC 96 Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAT AAG GAT GCA TAT GCT GCA AGT GCGGGG CGC CAG GAT ATGTCC CAA 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp MetSer Gln 35 40 45 GAC CCG AAG AAA TTT ACCGAT CCT GTT ATG GAC GTT ATA CAT GAG ATG 192 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCA CCA CTT AAA 207Ala Pro Pro Leu Lys65 [0066] array number: -- die-length [of 24 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- coxsackievirus A16 Stock name : the description description of 4057/81 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGG TCA CAG GTC TCC ACT CAG CGG TCT GGG of the gene field which carries out the code of the ****4 determined protein of information Coxsackie A group virus 16 mold besides approach:E TCA CAT GAG AAC TCA 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCT GCA TCG GAG GGT TCA ACT ATA AAT TAC ACA ACC ATA AAT TAC 96 Asn Ser Ala Ser Glu Gly Ser ThrIle Asn Tyr Thr ThrIle Asn Tyr 20 25 30 TAT AAG GAT GCA TAT GCT GCA AGT GCG GGG CGC CAG GAT ATG TCC CAA 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met SerGln 35 40 45 GAC CCGAAG AAA TTT ACC GAT CCT GTC ATG GAC GTT ATA CAT GAG ATG 192 Asp Pro Lys LysPhe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCA CCA CTC AAA 207Ala Pro Pro Leu Lys65 [0067] array number: -- die-length [of 25 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 16 shares of coxsackievirus A name: -- the description description of 0216/86 array The notation:CDS existence location to express : 1 -207 description The 1-207th base sequence array ATG GGG TCA CAG GTC TCC ACT CAG SGG TCT GGG of the gene field which carries out the code of the ****4 determined protein of information Coxsackie A group virus 16 mold besides approach:E TCA CAC GAA AAC TCA48 Met Gly Ser Gln Val Ser Thr Gln Xaa Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCT GYA TCG glass fiber reinforced gypsum GGT ACA WCT ATA AAT TAC ACA CCC ATA AAT TAC 96 Asn Ser Xaa Ser Xaa Gly Thr Xaa Ile Asn Tyr Thr ProIle Asn Tyr 20 25 30 TAT AAG GAT GCA TAT GCT GCA AGT GCG GGACGM CAG GAT ATGTCC CAG 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp MetSer Gln 35 40 45 GAC CCG AAG AAA TTC ACCGAT CCT GTC ATG GAC GTT ATA CAT GAG ATG 192 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCA CCG CTC AAA 207Ala Pro Pro Leu Lys65 [0068] array number: -- die-length [of 26 arrays]: -- mold [of 207 arrays]: -- number [of nucleic-acid chains]: -- single-strand topology: -- class [of straight chain-like array]: -- cDNA to genomic RNA origin living thing name: -- 16 shares of coxsackievirus A name: -- notation:CDS existence location: showing the

description description of 0241/91 array -- the 1-207th base sequences of the gene field which carries out the code of the ****4 protein of information Coxsackie A group virus 16 mold besides approach:E which determined 1 -207 description Array ATG GGG TCA CAG GTC TCC ACT CAA CGG TCT GGG TCA CAT GAG AAC TCA 48 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser 5 10 15 AAC TCA GCA TCA GAG GGT TCA ACT ATA AAT TAC ACA ACC ATA AAT TAC 96 Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr 20 25 30 TAT AAA GAT GCA TAT GCT GCG AGT GCG GGG CGC CAG GAT ATG TCC CAA 144 Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln 35 40 45 GAT CCG AAG AAA TTT ACC GAT CCT GTT ATG GAT GTT ATA CAC GAG ATG 192 Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met 50 55 60 GCT CCA CCA CTC AAA 207Ala Pro Pro Leu Lys65 [0069] array number: -- die-length [of 27 arrays]: -- mold [of 16 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array A part of information enterovirus of 5'untranslation region besides a synthetic DNA Array array CTACTTTGGG TGTCCG which has a complementarity in antigenomic sense RNA 16 [0070] array number: -- die-length [of 28 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array A part of gene field which carries out the code of the ****2 protein of information enterovirus besides a synthetic DNA Array which has a complementarity in genomic sense RNA. Q of the 6th base number shows T or C during an array, and N of the 18th base number shows A, C, G, or T.

Array GGTAQTTC ACCACCANCC 20 [0071] array number: -- die-length [of 29 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array A part of information enterovirus of 5'untranslation region besides a synthetic DNA Array array CATCTTTGGG TGTCCGTGTT which has a complementarity in antigenomic sense RNA 20 [0072] array number: -- die-length [of 30 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array A part of gene field which carries out the code of the VP2 protein of information enterovirus besides a synthetic DNA genomic sense Array array TCAGGCAACT TCCACCACCA which has a complementarity in RNA 20 [0073] array number: -- die-length [of 31 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 118-137th of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array AGGCTCTGTT TGCCTGCTGT which has a complementarity in antigenomic sense RNA 20 [0074] array number: -- die-length [of 32 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 118-137th of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array ATATCCTGGC GCCCCGCACT which has a complementarity in antigenomic sense RNA 20 [0075] array number: -- die-length [of 33 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 122-141st of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array TTTGAGGCTC TGTTTGCCTG which has a complementarity in antigenomic sense RNA 20 [0076] array number: -- die-length [of 34 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 122-141st of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array GGACATATCC TGGCGCCCCG which has a complementarity in antigenomic sense RNA 20 [0077] array number: -- die-length [of 35 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 181-200th of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array GGCGCTGCCA TTTCAGTGAA which has a complementarity in antigenomic sense RNA 20 [0078] array number: -- die-length [of 36 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 181-200th of the gene which

carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array GGTGGAGCCA TCTCATGTAT which has a complementarity in antigenomic sense RNA 20 [0079] array number: -- die-length [of 37 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 173-192nd of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array which has a complementarity in antigenomic sense RNA. Q of the 19th base number shows C or T during an array.

Array CATTTTCAGTG AAAATRTCQT 20 [0080] array number: -- die-length [of 38 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 173-192nd of the gene field which carries out the code of the ****4 protein of information enterovirus besides a synthetic DNA Array array CATCTCCTGT ATAACRTCCA which has a complementarity in antigenomic sense RNA 20 [0081] array number: -- die-length [of 39 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 118-137th of the gene field which carries out the code of ****4 of information enterovirus besides a synthetic DNA Array array ATATCTTGAC GCCCAGCGCT which has the complementarity of antigenomic sense RNA 20 [0082] array number: -- die-length [of 40 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 172-191st of the gene field which carries out the code of ****4 of information enterovirus besides a synthetic DNA Array which has the complementarity of antigenomic sense RNA. Q of the 3rd base number shows C or T during an array.

Array ATQTCATGTA TAACRTCCAT 20 [0083] array number: -- die-length [of 41 arrays]: -- mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array The 172-191st of the gene field which carries out the code of ****4 of information enterovirus besides a synthetic DNA Array which has the complementarity of antigenomic sense RNA. Q of the 18th base number shows C or T during an array.
Array ATTTTCAGTGA AAATRTCQTT 20

[Translation done.]

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- 1.This document has been translated by computer. So the translation may not reflect the original precisely.
- 2.**** shows the word which can not be translated.
- 3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.

[Drawing 2] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.

[Drawing 3] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.

[Drawing 4] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.

[Drawing 5] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.

[Drawing 6] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.

[Drawing 7] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.

[Drawing 8] It is drawing showing the hybridization of a CA16 unique probe (the array number 32, the array number 38, and array number 39) and Magnification DNA.

[Drawing 9] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.

[Drawing 10] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.

[Drawing 11] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.

[Drawing 12] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.

[Drawing 13] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.

[Drawing 14] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.

[Drawing 15] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.

[Drawing 16] It is drawing showing the hybridization of an EV71 unique DNA probe (the array number 31 and array number 41) and Magnification DNA.

[Drawing 17] It is drawing showing the joint location of a CA16 unique DNA probe and an EV71 unique DNA probe in the location of the magnification gene field of enterovirus, and the magnitude list of Magnification DNA. The location in the gene field where the figure in an array number and () carries out the code of the ****4 protein in two digits, and the figure in [] show the magnitude (bp) of a DNA fragment among drawing.

[Translation done.]

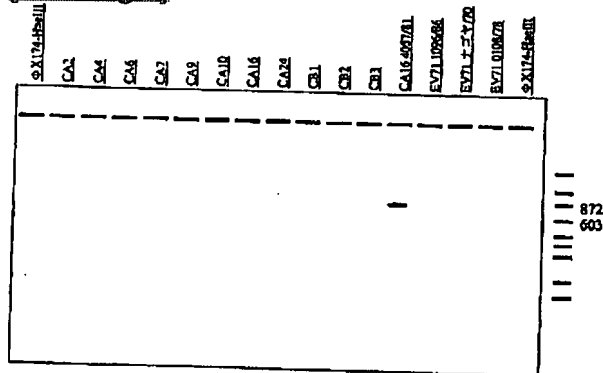
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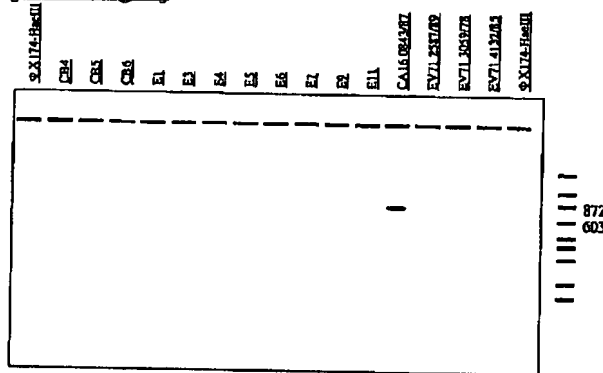
1. This document has been translated by computer. So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3. In the drawings, any words are not translated.

DRAWINGS

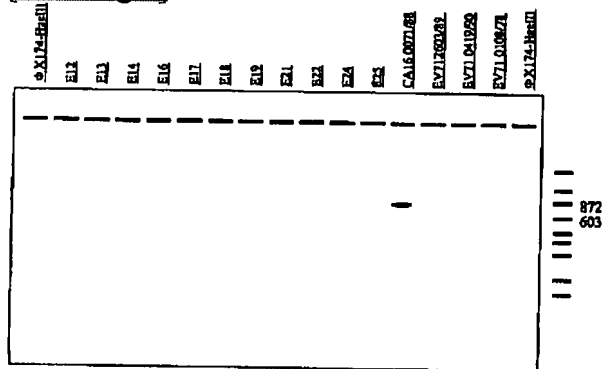
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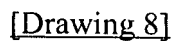
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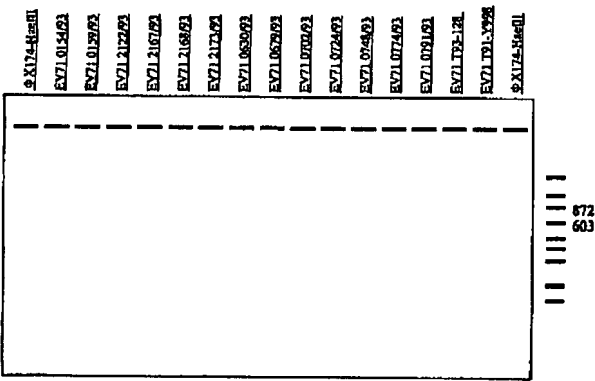


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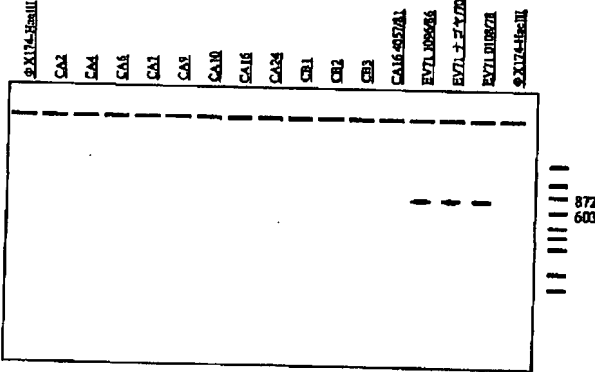


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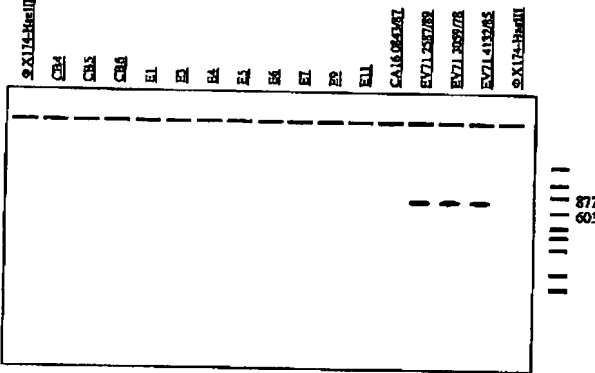




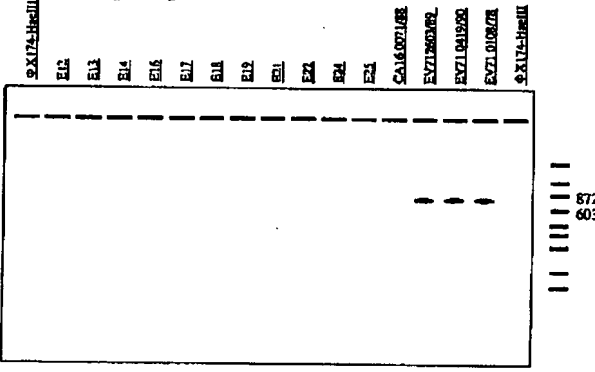
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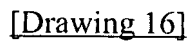
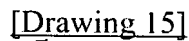
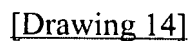
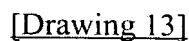
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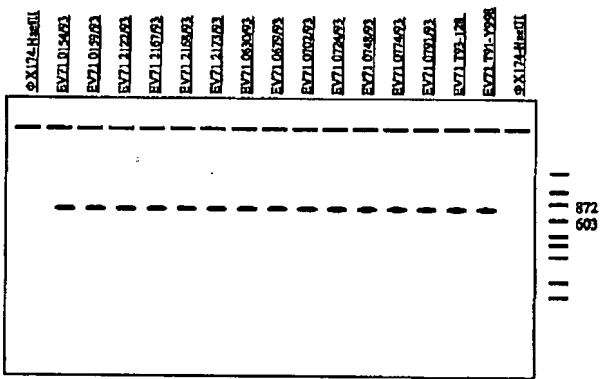


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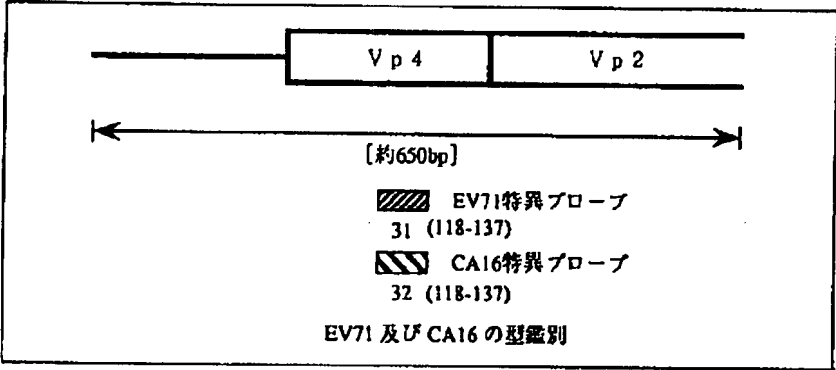
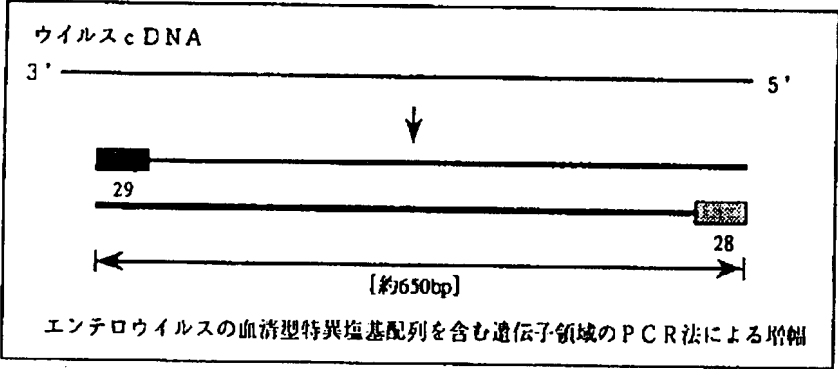
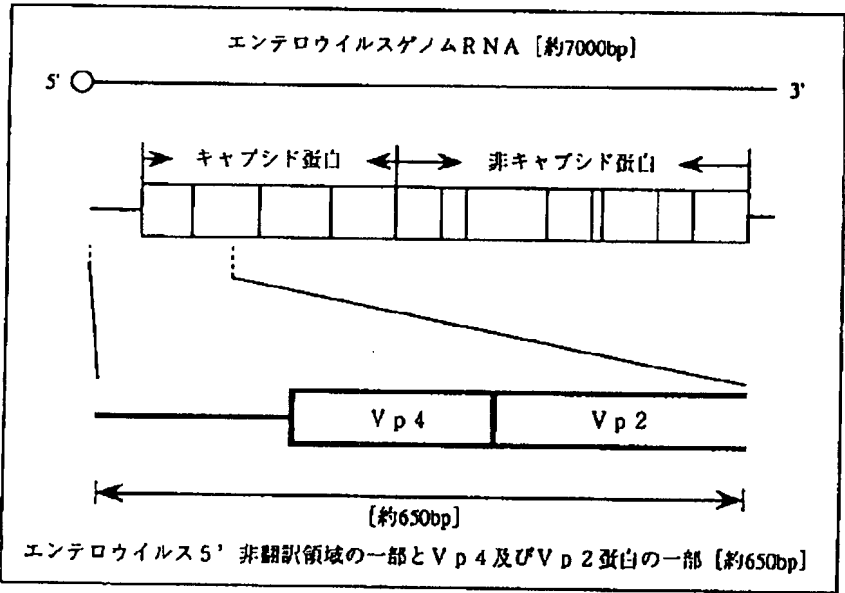


[Drawing 12]





[Drawing 17]



[Translation done.]

(19) 日本国特許庁 (J P)

(12) 公開特許公報 (A)

(11) 特許出願公開番号

特開平8-173195

(43) 公開日 平成8年(1996)7月9日

| (51) Int. Cl. ⁴ | 識別記号 | 片内整理番号 | P I | 技術表示箇所 |
|------------------------------|-------|---------|----------------|---------|
| C 1 2 Q 1/68 | A | 9453-4B | | |
| C 1 2 N 15/00 | Z N A | | | |
| C 1 2 Q 1/70 | | 9453-4B | | |
| | | 9162-4B | C 1 2 N 15/ 00 | Z N A A |
| 審査請求 未請求 請求項の数6 O L (全 28 頁) | | | | |

| | | | |
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| (33) 優先権主張国 | 日本 (J P) | (74) 代理人 | 弁理士 津国 肇 (外1名) |
| | | 最終頁に続く | |

(54) 【発明の名称】 エンテロウイルス71型及びコクサッキーA群ウイルス16型の型鑑別方法並びにそれに用いるDN
Aプローブ及びDNA断片

(57) 【要約】

【目的】 高い精度でエンテロウイルス71型 (EV71) とコクサッキーA群ウイルス16型 (CA16) の型鑑別を可能にする。

【解決手段】 (i) EV71及びCA16の5'非翻訳領域の一部とエンテロウイルスの各血清型に特異的なDNA配列を持つVP4及びVP2蛋白の一部をコードするDNA領域を増幅し、(ii) 該増幅DNA中のVP4蛋白をコードするDNA領域のDNA配列を決定し、(iii) 該DNA配列中のEV71及びCA16の各血清型に特異的なDNA配列に基づき、該DNA配列に各々相補性を有するDNA配列をそれぞれ設計してDNAプローブとし、(iv) 該DNAプローブと上記(i)の方法により得られる増幅DNAとの結合能を解析することによりEV71とCA16の型を鑑別する方法。

(2)

特開平8-173195

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【特許請求の範囲】

【請求項1】 (i) エンテロウイルス71型及びコクサッキーA群ウイルス16型の、5' 非翻訳領域の一部とエンテロウイルスの各血清型に特異的なDNA配列を持つVp4及びVp2蛋白の一部をコードするDNA領域を増幅し、(ii) 該増幅DNA中のVp4蛋白をコードするDNA領域のDNA配列を決定し、(iii) 該DNA配列中のエンテロウイルス71型及びコクサッキーA群ウイルス16型の各血清型に特異的なDNA配列に基づき、該DNA配列に各々相補性を有するDNA配列をそれぞれ設計してDNAプローブとし、(iv) 該DNAプローブと上記(i)の方法により得られる増幅DNAとの結合能を解析することを特徴とするエンテロウイルス71型及びコクサッキーA群ウイルス16型の型鑑別方法。

【請求項2】 増幅DNA中のエンテロウイルス71型のVp4蛋白をコードするDNA領域と相補性を有するDNA配列が、配列番号31、配列番号33、配列番号35、配列番号37又は配列番号41で示されるDNA配列であり、増幅DNA中のコクサッキーA群ウイルス16型のVp4蛋白をコードするDNA領域と相補性を有するDNA配列が、配列番号32、配列番号34、配列番号36、配列番号38、配列番号39又は配列番号40で示されるDNA配列である、請求項1記載の方法。

【請求項3】 配列番号31、配列番号33、配列番号35、配列番号37又は配列番号41で示されるDNA配列を含む、エンテロウイルス71型のVp4蛋白をコードするDNA領域の増幅DNAと相補性を有するDNAプローブ。

【請求項4】 配列番号32、配列番号34、配列番号36、配列番号38、配列番号39又は配列番号40で示されるDNA配列を含む、コクサッキーA群ウイルス16型のVp4蛋白をコードするDNA領域の増幅DNAと相補性を有するDNAプローブ。

【請求項5】 エンテロウイルス71型のVp4蛋白であって、そのアミノ酸配列中に配列番号1で示されるアミノ酸配列を含む蛋白をコードするDNA断片。

【請求項6】 コクサッキーA群ウイルス16型のVp4蛋白であって、そのアミノ酸配列中に配列番号2で示されるアミノ酸配列を含む蛋白をコードするDNA断片。

【発明の詳細な説明】

【0001】

【発明が属する技術分野】 本発明は、エンテロウイルスに属するエンテロウイルス71型（以下これを「EV71」と略記することがある）及びコクサッキーA群ウイルス16型（以下これを「CA16」と略記することがある）の鑑別方法、それに用いるDNAプローブ、並びにエンテロウイルス71型及びコクサッキーA群ウイルス

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ス16型のVp4蛋白をコードする遺伝子領域内の塩基配列に関する。

【0002】

【従来の技術】 ヒコルナウイルス科 (Picornaviridae) に属するエンテロウイルス (Enterovirus) はおよそ70種類の血清型に分類されており、多彩な感染症を示すため、臨床症状から原因となるウイルスを推定することは困難である。そのため、病原体を確定するにはウイルスの分離同定が必要となる。しかし、現在のエンテロウイルス分離同定法は、培養法を用いてウイルスを分離し、同定のためには更に中和試験が必要である。そしてこれらのウイルスの分離培養には、2～4週間が必要である。更に、標準株の中和抗血清を使用した中和試験においては、血清型鑑別不可能な分離株が頻繁に出現する。これはエンテロウイルスの遺伝子が自然界ではきわめて高速度で変異をするためと考えられており、これらの解決には常に新鮮分離株を中和する抗血清の作製が必要となる。

【0003】 高感度、特異的にDNAを増幅するポリメラーゼ・チェーン・リアクション法 (Polymerase Chain Reaction 法、以下これを「PCR法」と略記する; Sakaki, Science, 230巻, p.1350-1354, 1985 年参照) が開発されてから、エンテロウイルスは、5' 非翻訳領域の塩基配列に相補的なプライマーを用いたPCR法や、5' 非翻訳領域内、Vp4とVp2蛋白をコードする遺伝子領域の塩基配列に相補性を有するプライマーを用いたPCR法で検出されている (Rotbart, H., S. J. Clinical Microbiology, 28, 438-442 (1990); Olive, D. M., S. J. General Virology, 71, 2141-2147 (1990))。

【0004】 さらに、PCRにより増幅されたDNAを用いたストリンジェント・リバーシブルハイブリダイゼーション法によるエンテロウイルスの型同定が報告されている (石古博昭等、臨床とウイルス、22 巻、ページ199-207 (1994))。しかし、本実験では、同一血清型の中でも年代や地域の違いによりターゲット遺伝子とプローブとの反応性が異なり、特定のウイルス株をプローブに選定することが困難であると予想される。エンテロウイルスによる疾患のなかで、手足口病はその患者から分離されるウイルスがEV71とCA16で90%以上を占めるので、2種の血清型を迅速にかつ簡易に検出することにより手足口病の流行ウイルスを同定することが可能である。

【0005】

【発明が解決しようとする課題】 本発明は、EV71とCA16を高い精度で型鑑別できる方法の提供を目的とする。

【0006】

【課題を解決するための手段】 本発明者らは、上記目的を達成すべく鋭意検討を重ねた結果、EV71及びCA

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16のVp4蛋白をコードする遺伝子領域の塩基配列中に、各血清型に特異的な塩基配列があり、該塩基配列を用いてEV71とCA16が高い精度で鑑別可能であることを見出し、本発明を完成するに至った。即ち本発明は、下記1～6に記載のエンテロウイルス71型及びコクサッキーA群ウイルス16型の型鑑別方法、それに用いるDNAプローブ及びDNA断片を提供するものである。

【0007】1. (i) エンテロウイルス71型及びコクサッキーA群ウイルス16型の、5' 非翻訳領域の一部とエンテロウイルスの血清型に特異的なDNA配列を持つVp4及びVp2蛋白の一部をコードするDNA領域を増幅し、(ii) 該増幅DNA中のVp4蛋白をコードするDNA領域のDNA配列を決定し、(iii) 該DNA配列中のエンテロウイルス71型及びコクサッキーA群ウイルス16型の各血清型に特異的なDNA配列に基づき、該DNA配列に各々相補性を有するDNA配列をそれぞれ設計してDNAプローブとし、(iv) 該DNAプローブと上記(i)の方法により得られる増幅DNAとの結合能を解析することを特徴とするエンテロウイルス71型及びコクサッキーA群ウイルス16型の型鑑別方法。

【0008】2. 増幅DNA中のエンテロウイルス71型のVp4蛋白をコードするDNA領域と相補性を有するDNA配列が、配列番号31、配列番号33、配列番号35、配列番号37又は配列番号41で示されるDNA配列であり、増幅DNA中のコクサッキーA群ウイルス16型のVp4蛋白をコードする遺伝子領域と相補性を有するDNA配列が、配列番号32、配列番号34、配列番号36、配列番号38、配列番号39又は配列番号40で示されるDNA配列である、上記1記載の方法。

3. 配列番号31、配列番号33、配列番号35、配列番号37又は配列番号41で示されるDNA配列を含む、エンテロウイルス71型のVp4蛋白をコードするDNA領域の増幅DNAと相補性を有するDNAプローブ。

【0009】4. 配列番号32、配列番号34、配列番号36、配列番号38、配列番号39又は配列番号40で示されるDNA配列を含む、コクサッキーA群ウイルス16型のVp4蛋白をコードするDNA領域の増幅DNAと相補性を有するDNAプローブ。

5. エンテロウイルス71型のVp4蛋白であって、そのアミノ酸配列中に配列番号1で示されるアミノ酸配列を含む蛋白をコードするDNA断片。

6. コクサッキーA群ウイルス16型のVp4蛋白であって、そのアミノ酸配列中に配列番号2で示されるアミノ酸配列を含む蛋白をコードするDNA断片。以下、本発明について更に詳細について説明する。

【0010】本発明の実施においては、特に指示されな

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い限り当該分野の技術範囲内にある分子生物学、微生物学、組換えDNA、及び免疫学に於ける従来の手法が採用される。このような手法は文献中に詳しく説明されている。例えば次のような文献を参照されたい。Maniatis, Fritsch及び Sambrook, MOLECULAR CLONING; A LABORATORY MANUAL (1982); Kevin Struhlら, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, 1巻及び2巻; 村松正實編, ラボマニュアル遺伝子工学 (1990); 村松正實 岡山博人編, 遺伝子工学ハンドブック, 実験医学 別冊 (1991); 東京大学医科学研究所制御研究部編, 細胞工学実験プロトコル (1991)。

【0011】1. エンテロウイルス71型及びコクサッキーA群ウイルス16型の5' 非翻訳領域の一部とエンテロウイルスの血清型に特異的な塩基配列を持つVp4及びVp2蛋白の一部をコードする遺伝子領域の増幅エンテロウイルス71型及びコクサッキーA群ウイルス16型の5' 非翻訳領域の一部とエンテロウイルスの血清型に特異的な塩基配列を持つVp4及びVp2蛋白の一部をコードする遺伝子領域(以下これを「エンテロウイルスの血清型特異的塩基配列を含む遺伝子領域」と略称することがある)の増幅は、次のとおり行うことができる。

【0012】まず、血清型が既知のエンテロウイルス分離培養株、継代培養されている血清型が既知のエンテロウイルス標準株等から常法によりRNAを抽出し、この抽出RNAから、逆転写酵素を用いてcDNAを作製する。このcDNAにエンテロウイルスの血清型特異的塩基配列を含む遺伝子領域の上流の型共通部分及び下流の型共通部分に相補性を有するオリゴヌクレオチドをプライマーとして加えて、エンテロウイルス71型及びコクサッキーA群ウイルス16型の5' 非翻訳領域、Vp4とVp2をコードする遺伝子領域を含む長さが約650塩基の遺伝子DNA領域を増幅する。

【0013】遺伝子の増幅は、通常用いられるPCR法(このPCR法の詳細については、特開昭61-274697号公報、特開昭62-281号公報、Sakaiら Science 239巻, p.487-491 参照)により容易に行うことができる。エンテロウイルスの血清型特異的塩基配列を含む遺伝子領域の増幅に際して、プライマーとして用いることができるオリゴヌクレオチド(以下これを「血清型共通プライマー」と略称することがある)としては、血清型特異的塩基配列を含む遺伝子領域の上流の型共通部分及び下流の型共通部分に相補性を有するオリゴヌクレオチドを同時に用いるのであれば、いかなるオリゴヌクレオチドであってもよい。それらの中で、好ましくは既知の血清型特異的塩基配列データをもとに、エンテロウイルスに特異的かつ種間で共通性の高い塩基配列を5' 非翻訳領域(上流の型共通部分)とVp2領域(下流の型共通部分)に設定し、その塩基配列に基づいて化学合成したオリゴヌクレオチドをプライマーとして用いる

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のが適当である。

【0014】上記プライマーの具体例としては、D.M. Olive et al, Journal of General Virology 71巻、ページ 2141-2147 (1990) に記載されている配列番号27及び配列番号28で示されるオリゴヌクレオチド、本願発明者らが先に提案した特開平6-311900号明細書に記載されている配列番号29及び配列番号30で示されるオリゴヌクレオチドを挙げることができる。これらプライマーの中で、5'末端側のプライマーとして配列番号29で示されるオリゴヌクレオチド、3'末端側のプライマーとして配列番号28で示されるオリゴヌクレオチドを同時に用いるのが適当である。

【0015】上記したプライマーは、それ自体既知の通常用いられる核酸合成装置、例えばアプライド・バイオシステムズ (Applied Biosystems) 社製、モデル381-A DNA合成機等を用いる固相合成法により、容易に化学合成することができる。上記増幅DNAは、常法、例えば、アガロースゲル電気泳動法により分離し、DNAバンドとして検出することができ、これによりエンテロウイルス71型及びコクサッキーA群ウイルス16型由来の遺伝子DNAを確認することができる。

【0016】2. 増幅DNA中のVp4蛋白をコードする遺伝子領域の塩基配列の決定
上記増幅DNAの塩基配列は、例えば、アプライド・バイオシステムズ社製、モデル373-A オートシーケンサーを用い、Dye DeoxyTM ターミネーター法により決定することができる。決定された塩基配列中の翻訳開始コドン及びエンテロウイルスの既知のVp4蛋白及びVp2蛋白を切断するアミノ酸配列に対応する塩基配列【蛋白質 核酸酵素、137巻、14号、ページ2609-2618 (1992) 参照】を探索することにより、Vp4蛋白をコードする遺伝子領域の塩基配列を決定することができる。

【0017】かくして得られるEV71のVp4蛋白をコードする遺伝子領域の配列を配列番号3～配列番号21に、そのアミノ酸配列の組合せを配列番号1に示す。また、CA16のVp4蛋白をコードする遺伝子領域の配列を配列番号22～配列番号26に、そのアミノ酸配列の組合せを配列番号2に示す。上記塩基配列を包含する本発明のDNA断片は、EV71及びCA16分離株のRNAから合成されたcDNAのみならず、通常用いられるDNA合成装置、例えば上記アプライド・バイオシステムズ社製、モデル381-A DNA合成機を用いて合成されたものであってよい。

【0018】3. DNAプローブの設計及び合成
エンテロウイルス71型の特異的な塩基配列に相補性を有するDNAプローブ（以下これを「EV71特異プローブ」と略称することがある）の塩基配列は、前記配列番号3～配列番号21で示される配列中のEV71に特異的な配列を解析することにより設計することができ

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る。同様に、コクサッキーA群ウイルス16型の特異的な塩基配列に相補性を有するDNAプローブ（以下これを「CA16特異プローブ」と略称することがある）の塩基配列は、前記配列番号22～配列番号26で示される配列中の、CA16に特異的な配列を解析することにより設計することができる。

【0019】かくして得られるEV71特異プローブとしては、例えば、配列番号31、配列番号33、配列番号35、配列番号37又は配列番号41で示されるオリゴヌクレオチド、CA16特異プローブとしては、例えば、配列番号32、配列番号34、配列番号36、配列番号38、配列番号39又は配列番号40で示されるオリゴヌクレオチドを挙げることができる。上記した血清型特異プローブのうち、好ましくは、EV71特異プローブとして配列番号31及び配列番号41、CA16特異プローブとして配列番号32、配列番号39及び配列番号40で示されるオリゴヌクレオチドを挙げることができる。上記DNAプローブは、それ自体既知の通常用いられる核酸合成装置、例えば、アプライド・バイオシステムズ社製、モデル381-A DNA合成機等を用いる固相合成法により、容易に化学合成することができる。

【0020】4. エンテロウイルス71型及びコクサッキーウイルス16型の型鑑別

上記した、血清型特異プローブを用いて、次のとおり、EV71及びCA16を鑑別することができる。まず、診察時に採取した臨床検体からのウイルス分離培養株から、常法によりウイルスRNAを抽出する。該抽出RNAからcDNAを合成後、前記血清型共通プライマーを用いて、エンテロウイルスの5'非翻訳領域の一部、Vp4及びVp2蛋白の一部をコードする遺伝子領域を、前記PCR法により増幅する。増幅DNAの検出は、アガロース電気泳動により増幅DNAを分離し、適当な染色剤、例えばエチジウム・ブロマイド等で染色後、紫外線照射等により染色させ、そのDNAバンドを確認することにより行うことができる。

【0021】EV71とCA16の型鑑別は、前記血清型共通プライマーを用いて増幅したDNA断片と、前記血清型特異DNAプローブ、すなわちEV71の検出には、例えば配列番号31、配列番号33、配列番号35、配列番号37及び配列番号41で示される配列を、またCA16の検出には、例えば配列番号32、配列番号34、配列番号36、配列番号38、配列番号39及び配列番号40で示される配列を有するオリゴヌクレオチドを標識することにより作製したDNAプローブとを、固相上で常法によりハイブリダイズさせ、血清型特異DNAプローブの種類を検出解析することにより行うことができる。

【0022】固相上へのDNAの結合は、それ自体既知の通常用いられる方法、例えば、増幅DNAを変性後、

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適当な固相、例えばナイロンメンブレンに、常法により化学結合させることにより行うことができるが、好ましくは、増幅DNAを確認するために使用した上記アガロースゲルを変性後、中和し、例えばナイロンメンブレンに増幅DNAをトランスファー後固定する方法（以下これを「サザンブロッティング法」と略称することがある）が挙げられる。

【0023】DNAプローブの標識は、5'末端のリン酸化反応により〔 γ - 32 P〕ATP、T4ポリヌクレオチドキナーゼ（東洋紡績社製）を用いて行うことができる。また、標識物の検出は、標識されたDNAプローブとのハイブリダイゼーションを行ったナイロンメンブレンのオートラジオグラフィーを行い、各々のDNAプローブとのハイブリッド形成の有無を調べることにより行うことができる。

【0024】上記した、本発明で用いられるエンテロウイルスの血清型特異的DNA配列を含む遺伝子領域、即ち、エンテロウイルスの5'非翻訳領域の一部とVp4及びVp2蛋白の一部をコードする領域、血清型共通プライマー、DNAプローブ、該プライマーを用いて増幅*20

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*可能なDNA断片等の一例を、図17に示す。図中、2桁の数字は配列番号、（ ）内の数字はDNAプローブが結合するVp4蛋白をコードする遺伝子領域の位置（塩基対番号）、[]内の数字は増幅されるDNA断片の大きさ、bpは塩基対を示す。

【0025】

【実施例】次に実施例を挙げて本発明をさらに詳細に説明するが、下記の実施例は本発明について具体的な認識を得る一助としてのみ挙げたものであり、これによって本発明の範囲は何等限定されるものではない。

【0026】実施例1 EV71とCA16分能培養株のシーケンス

（A）使用微生物

下記の、患者より分離され特異抗血清を用いた中和試験により血清型が同定されたエンテロウイルス分能株及び標準株を用いて実験を行った。ここで用いた標準株は、国立予防衛生研究所において継代培養されているウイルス標準株である。

【0027】

（1）エンテロウイルス分離株

| 株名（血清型） | 分離時期 |
|-----------------------|-------|
| コクサッキーA群ウイルス16型（CA16） | |
| 1547/79 | 1979年 |
| 4057/81 | 1981年 |
| 0216/86 | 1986年 |
| 0241/91 | 1991年 |
| エンテロウイルス71型（EV71） | |
| ナゴヤ/70 | 1970年 |
| 0108/78 | 1978年 |
| 3059/78 | 1978年 |
| 3359/83 | 1983年 |
| 4132/85 | 1985年 |
| T86236a/86 | 1986年 |
| 1091/89 | 1989年 |
| 0445/90 | 1990年 |
| 0004/78 | 1978年 |
| 1096/86 | 1986年 |
| 0872/89 | 1989年 |
| 2587/89 | 1989年 |
| 2603/89 | 1989年 |
| 0375/90 | 1990年 |
| 2136/90 | 1990年 |
| 2398/90 | 1990年 |
| 4094/90 | 1990年 |
| 0419/90 | 1990年 |

【0028】（2）標準株

| 株名（血清型） | 分離時期 |
|--------------|-----------|
| コクサッキーA群ウイルス | 16型（CA16） |
| エンテロウイルス | 71型（EV71） |

【0029】（B）RNAの抽出

上記ウイルス液から、スマイテストRキット（住友金属社製）にてRNAを抽出し、イソプロパノール沈殿を行った。

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(C) cDNAの合成

上記(B)項で得たRNAを鋳型としてリバーストランスクリプターゼ(GIBC0/BRL社製)を用いて、各ウイルスに由来するcDNAを合成した。

(D) PCR用のプライマーの合成

配列番号28及び配列番号29で示されるオリゴヌクレオチドをホスホアミダイト(Phosphoramidite)法により、アプライド・バイオシステムズ社製、モデル381-A DNA合成機を用いて合成した。上記オリゴヌクレオチドをOPC[®]カートリッジを用いて精製し、PCRのプライマーとして使用した。

【0030】(E) 標的DNAの増幅(PCR法)

反応液は、終濃度を10mM Tris・HCl (pH8.0)、50mM KCl、0.001%ゼラチン、0.55mM MgCl₂、0.05mM dNTPs、2.5%ホルムアミドとした溶液に、上記オリゴヌクレオチド合成プライマー(配列番号28及び配列番号29;各50μM)各0.1μl、前記(C)項で合成されたエンテロウイルスcDNA 100ng~1μg、及びTaqポリメラーゼ(日本ロッッシュ社製)0.125μl(0.625U)を加え、計50μlとしたものを調製した。PCRは、95℃30秒、55℃30秒の反応条件で、14サイクルでアンプリフィケーションシステム(Amplification System; シータス社)を用いて標的DNAを増幅した。

【0031】さらに、上記溶液に、終濃度を10mM Tris・HCl (pH8.0)、50mM KCl、0.001%ゼラチン、2.35mM MgCl₂、0.15mM dNTPs、2.5%ホルムアミドとした溶液に、上記オリゴヌクレオチド合成プライマー(配列番号28及び配列番号29;各50μM)各1.0μl、Taqポリメラーゼ0.25μl(1.25U)を加えた計50μlの溶液を加え、95℃30秒、55℃30秒、72℃

*℃45秒、40サイクルで増幅した。

【0032】(F) ゲル電気泳動法による増幅DNAの確認

3.0%のアガロースゲルにエチジウムブロマイドを0.5μg/ml加え、上記(E)項で増幅したDNAの電気泳動を行った。泳動後、254nmの紫外線を照射し、エチジウムブロマイドの発色反応によりDNAバンドを検出し、エンテロウイルスの5'非翻訳領域の一部、Vp4とVp2蛋白の一部をコードする遺伝子領域に由来する約650塩基の標的DNAバンドを検出した。

【0033】(G) Vp4領域の塩基配列の決定

上記(F)で確認されたPCR産物は、Centricon-100(アミコン社製)を用いて精製後、Dye Deoxy[®]ターミネーター法によりCycle Sequencing反応を行い、アプライド・バイオシステムズ社製モデル373-A DNAシーケンサーを用い、塩基配列を決定した。決定された塩基配列中の翻訳開始コドン及びエンテロウイルスの既知のVp4蛋白及びVp2蛋白を切断するアミノ酸配列に対応する塩基配列を探索することにより、Vp4蛋白をコードする遺伝子領域の塩基配列を決定した。かくして得られたEV71のVp4蛋白をコードする配列を配列番号3~配列番号21に、CA16のVp4蛋白をコードする配列を配列番号22~配列番号26に示す。

【0034】実施例2 EV71及びCA16の型鑑別

(A) 使用微生物

(1) 標準株

国立予防衛生研究所において継代培養されている下記39種類の血清型エンテロウイルス標準株を用いて実験を行った。これらのエンテロウイルスは、いずれも特異抗血清を用いた中和試験で血清型が同定されている標準株である。

【0035】

| 株名(血清型) | 2型 | 略号 |
|--------------|------|------|
| コクサッキーA群ウイルス | 2型 | CA2 |
| " | 4 " | CA4 |
| " | 6 " | CA6 |
| " | 7 " | CA7 |
| " | 9 " | CA9 |
| " | 10 " | CA10 |
| " | 16 " | CA16 |
| " | 24 " | CA24 |
| コクサッキーB群ウイルス | 1型 | CB1 |
| " | 2 " | CB2 |
| " | 3 " | CB3 |
| " | 4 " | CB4 |
| " | 5 " | CB5 |
| " | 6 " | CB6 |
| エコーウイルス | 1型 | E1 |
| " | 3 " | E3 |

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| | | |
|----------|------|------|
| " | 4 " | E4 |
| " | 5 " | E5 |
| " | 6 " | E6 |
| " | 7 " | E7 |
| " | 9 " | E9 |
| " | 11 " | E11 |
| " | 12 " | E12 |
| " | 13 " | E13 |
| " | 14 " | E14 |
| " | 16 " | E16 |
| " | 17 " | E17 |
| " | 18 " | E18 |
| " | 19 " | E19 |
| " | 21 " | E21 |
| " | 22 " | E22 |
| " | 24 " | E24 |
| " | 25 " | E25 |
| " | 30 " | E30 |
| エンテロウイルス | 70型 | EV70 |
| " | 71 " | EV71 |
| ポリオウイルス | 1型 | PV1 |
| " | 2 " | PV2 |
| " | 3 " | PV3 |

【0036】(2) エンテロウイルス分離株 *いて実験を行った。
 下記の、患者より分離され特異抗血清を用いた中和試験 【0037】
 により血清型が同定されたエンテロウイルス分離株を用*

| 株名 | 分離時期 |
|-----------------------|-------|
| コクサッキーA群ウイルス16型(CA16) | |
| 4057/81 | 1981年 |
| 0843/87 | 1987年 |
| 0071/88 | 1988年 |
| 1045/90 | 1990年 |
| 1051/90 | 1990年 |
| 0241/90 | 1990年 |
| 2500/92 | 1992年 |
| エンテロウイルス71型(EV71) | |
| ナゴヤ/70 | 1970年 |
| 2105/73 | 1973年 |
| 2089/73 | 1973年 |
| 2146/73 | 1973年 |
| 2203/73 | 1973年 |
| 2377/73 | 1973年 |
| 2381/73 | 1973年 |
| 0108/78 | 1978年 |
| 3059/78 | 1978年 |
| 0193/78 | 1978年 |
| 0297/78 | 1978年 |
| 0333/78 | 1978年 |
| 0761/82 | 1982年 |
| FE-27 | 1982年 |

| 13 | (8) | 特開平8-173195 | 14 |
|----------|-----|-------------|----|
| 0272/83 | | 1983年 | |
| FE-18 | | 1983年 | |
| FE-20 | | 1983年 | |
| 4132/85 | | 1985年 | |
| 0866/86 | | 1986年 | |
| 0891/86 | | 1986年 | |
| 0948/86 | | 1986年 | |
| 1025/86 | | 1986年 | |
| 1027/86 | | 1986年 | |
| 1079/86 | | 1986年 | |
| 1096/86 | | 1986年 | |
| 1163/86 | | 1986年 | |
| 0460/87 | | 1987年 | |
| 0443/88 | | 1988年 | |
| 2587/89 | | 1989年 | |
| 2603/89 | | 1989年 | |
| 2136/90 | | 1990年 | |
| 2234/90 | | 1990年 | |
| 2398/90 | | 1990年 | |
| 0419/90 | | 1990年 | |
| 0445/90 | | 1990年 | |
| 0189/90 | | 1990年 | |
| 4094/90 | | 1990年 | |
| FE-45 | | 1990年 | |
| FE-46 | | 1990年 | |
| T91-Y563 | | 1991年 | |
| 1264/92 | | 1992年 | |
| 2326/92 | | 1992年 | |
| 2909/93 | | 1993年 | |
| 0089/93 | | 1993年 | |
| 0133/93 | | 1993年 | |
| 0136/93 | | 1993年 | |
| 0146/93 | | 1993年 | |
| 2200/93 | | 1993年 | |
| 0154/93 | | 1993年 | |
| 0159/93 | | 1993年 | |
| 2122/93 | | 1993年 | |
| 2167/93 | | 1993年 | |
| 2168/93 | | 1993年 | |
| 2173/93 | | 1993年 | |
| 0565/93 | | 1993年 | |
| 0630/93 | | 1993年 | |
| 0679/93 | | 1993年 | |
| 0702/93 | | 1993年 | |
| 0724/93 | | 1993年 | |
| 0748/93 | | 1993年 | |
| 0774/93 | | 1993年 | |
| 0791/93 | | 1993年 | |
| FE-57 | | 1993年 | |
| T93-128 | | 1993年 | |

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T93-133
T93-Y998

【0038】(B) RNA抽出とcDNA合成、及びエンテロウイルス遺伝子のPCRによる増幅とその検出
実施例1の(B)～(F)項で示したように、上記エンテロウイルス標準株及び分離株のRNAを抽出し、5'非翻訳領域の一部、Vp4とVp2蛋白の一部に対応する部分を増幅後、アガロースゲルを用いた電気泳動にて増幅を確認した。なお、マーカーとして ϕ X174ファージのHaeIII分解物を同時に泳動した。

【0039】(C) サザンブロット法

実施例2の(B)項で増幅DNAの確認のために使用したアガロースゲルを、アルカリ変性液(0.5M NaOH, 1.5M NaCl)で30分間処理後、中和液(1M Tris-HCl, 1.5M NaCl)で30分間入れゲルを中性に戻し、DNAを変性させた。さらに、常法により、サザンブロッティングを一晩行い、ナイロンメンブレンに移行したDNAを254nmの紫外線 $1.2 \times 10^4 \mu\text{Joules/cm}^2$ (UV-stratalinkerTM; ストラタジーン製)により固着させた。

【0040】(D) 血清型特異的プローブの設計と合成
実施例1(G)項で示したCA16及びEV71のVp4蛋白質をコードするDNA領域の塩基配列【配列番号3から配列番号26】を解析し、これらの配列からEV71特異プローブを配列番号31及び配列番号41、CA16特異プローブを配列番号32、配列番号39及び配列番号40のように設計し、実施例1(D)項に進んで合成した。上記DNAプローブの標識は、5'末端のリン酸化反応により $[\gamma\text{-}^{32}\text{P}] \text{ATP}$ 、T4ポリヌクレオチドキナーゼ(東洋紡績社製)を用いて行った。

【0041】(E) ハイブリダイゼーション

上記実施例2(C)項で作製したメンブレンは、反応液として1.0M NaCl, 50mM Tris-HCl (pH7.5), 0.5% PVP, 0.2%ヘパリン, 1mM EDTA, 2% SDSを用い、50℃で1時間処理し、プレハイブリダイゼーションを行った。ハイブリダイゼーションは、上記ハイブリダイゼーション溶液に、実施例2の(D)項で合成した $[\gamma\text{-}^{32}\text{P}] \text{ATP}$ 標識DNAプローブを、 10^5 cpm/ml加え、50℃、4時間反応させた。メンブレンを $5 \times \text{SSC}-0.1\% \text{SDS}$ 40

配列

```

Met Gly Gaa Gln Val Ser Hhh Gln Ini Ser Gly Ser His Glu Asn Ser
      5              10              15
Jij Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Kkk Tyr
      20              25              30
Tyr Lll Mmm Ser Tyr Ala Ala Thr Ala Gly Mnn Gln Ser Leu Ooo Gln
      35              40              45
Ppp Pro Asp Lys Qaa Rrr Sss Pro Val Lys Asp Ile Phe Thr Glu Met
      50              55              60

```

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1993年
1993年

*0.1% SDS溶液中で、50℃で5分、2回洗浄し、非特異的に結合しているプローブを取り除いた。ナイロンメンブレンを乾燥後、ラップに包み、オートラジオグラフィを-80℃で45分間、さらに室温で15分間行った後、現像した。

【0042】かくして得られた、CA16特異的DNAプローブ(配列番号32、配列番号38及び配列番号39)と増幅DNAとのハイブリッドの形成を図1～図8に、EV71特異的DNAプローブ(配列番号31及び配列番号41)と増幅DNAとのハイブリッド形成を図9～図16に示す。図から明らかなとおり、EV71特異的DNAプローブにはすべてのEV71分離株が、CA16特異的DNAプローブにはすべてのCA16分離株が反応し、かつ、血清型特異的DNAプローブと各血清型のエンテロウイルス標準株から得られたDNAとの間での交差反応はなく、これは特異的な結合であることが判明した。上記のとおり、本発明の方法によれば、CA16とEV71が高精度に鑑別可能である。

【0043】

【配列表】

配列番号: 1

配列の長さ: 69

配列の型: アミノ酸

トポロジー: 直鎖状

他の情報

下記配列中、アミノ酸番号3番目のGaaは、Ser又はThrを示し、アミノ酸番号7番目のHhhは、Ala又はThrを示し、アミノ酸番号9番目のIniは、Arg又はXaaを示し、アミノ酸番号17番目のJijは、Asn又はXaaを示し、アミノ酸番号31番目のKkkは、Asn又はXaaを示し、アミノ酸番号34番目のLllは、Lys又はXaaを示し、アミノ酸番号35番目のMmmは、Asp又はXaaを示し、アミノ酸番号43番目のMnnは、Lys又はXaaを示し、アミノ酸番号47番目のOooは、Lys又はXaaを示し、アミノ酸番号49番目のPppは、Asp又はXaaを示し、アミノ酸番号53番目のQaaは、Phe又はXaaを示し、アミノ酸番号54番目のRrrは、Ala又はGly又はXaaを示し、アミノ酸番号55番目のSssは、Asn又はXaaを示す。

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Ala Ala Pro Leu Lys

65

【0044】配列番号: 2

配列の長さ: 69

配列の型: アミノ酸

トポロジー: 直鎖状

他の情報

下記配列中、アミノ酸番号9番目のAaaは、Arg又はXa*

配列

Met Gly Ser Gln Val Ser Thr Gln Aaa Ser Gly Ser His Glu Asn Ser

5 10 15

Asn Ser Bbb Ser Ccc Gly Ddd Eee Ile Asn Tyr Thr Fff Ile Asn Tyr

20 25 30

Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln

35 40 45

Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met

50 55 60

Ala Pro Pro Leu Lys

65

【0045】配列番号: 3

配列の長さ: 207

配列の型: 核酸

鎖の数: 一本鎖

トポロジー: 直鎖状

配列の種類: cDNA to genomic RNA

起源

生物名: enterovirus enterovirus type 71

配列

ATG GGC TCC CAG GTC TCC ACA CAG CGA TCC GGC TCG CAT CAG AAT TCC 48

Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser

5 10 15

AAC TCA GGC ACG GAA GGC TCC ACT ATA AAT TAC ACA ACC ATT AAT TAC 96

Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr

20 25 30

TAC AAA GAC TCG TAT GCT GGC ACT GCT GGA AAG CAA AGT CTC AAA CAA 144

Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln

35 40 45

CAT CCT GAC AAG TTT GCG AAC CCT GTG AAG GAC ATC TTT ACT GAA ATG 192

Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met

50 55 60

GCA GCG CCC TTA AAG 207

Ala Ala Pro Leu Lys

65

【0046】配列番号: 4

配列の長さ: 207

配列の型: 核酸

鎖の数: 一本鎖

トポロジー: 直鎖状

配列の種類: cDNA to genomic RNA

起源

* a を示し、アミノ酸番号19番目のBbbは、Ala 又はXa
 a を示し、アミノ酸番号21番目のCccは、Glu 又はXa
 a を示し、アミノ酸番号23番目のDddは、Ser 又はTh
 r を示し、アミノ酸番号24番目のEeeは、Thr 又はXa
 a を示し、アミノ酸番号29番目のFffは、Thr 又はPro
 を示す。

20※ 株名: BrCr

配列の特徴

特徴を表す記号: CDS

存在位置: 1-207

特徴を決定した方法: E

他の情報

エンテロウイルス71型のVp4蛋白をコードする遺伝

※ 子領域の1~207番目の塩基配列

生物名: enterovirus type 71

株名: ナゴヤ/70

配列の特徴

特徴を表す記号: CDS

存在位置: 1-207

特徴を決定した方法: E

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エンテロウイルス71型のVp4蛋白をコードする遺伝* *子領域の1~207番目の塩基配列
配列

```

ATG GGT TCA CAA GTG TCT ACT CAG CGG TCC GCG TCC CAC GAG AAT TCC 48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
      5              10              15
AAT TCA GCT ACA GAA GGT TCC ACC ATT AAT TAC ACT ACT ATC AAT TAT 96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
      20              25              30
TAC AAG GAC TCT TAT GCT GCG ACA GCA GCG AAG CAG AGC CTC AAA CAA 144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
      35              40              45
GAC CCT GAC AAG TTT GCG AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192
Asp Pro Asp Lys Phe Gly Asn Pro Val Lys Asp Ile Phe Thr Glu Met
      50              55              60
GCG GCG CCA CTG AAG 207
Ala Ala Pro Leu Lys
      65

```

【0047】配列番号: 5

※株名: 0108/78

配列の長さ: 207

配列の特徴

配列の型: 核酸

20 特徴を表す記号: CDS

鎖の数: 一本鎖

存在位置: 1-207

トポロジー: 直鎖状

特徴を決定した方法: E

配列の種類: cDNA to genomic RNA

他の情報

起源

エンテロウイルス71型のVp4蛋白をコードする遺伝

生物名: enterovirus type 71

※ 子領域の1~207番目の塩基配列

```

ATG GGT TCA CAA GTG TCT ACT CAG CGG TCC GCG TCC CAC GAG AAT TCT 48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
      5              10              15
AAT TCA GCT ACA GAA GCG TCC ACC ATC AAT TAC ACT ACC ATC AAC TAT 96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
      20              25              30
TAC AAG GAC TCT TAT GCT GCG ACA GCA GGT AAG CAG AGC CTC AAA CAA 144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
      35              40              45
GAC CCT GAC AAG TTT GCT AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
      50              55              60
GCG GCG CCA CTG AAG 207
Ala Ala Pro Leu Lys
      65

```

【0048】配列番号: 6

★株名: 3059/73

配列の長さ: 207

配列の特徴

配列の型: 核酸

特徴を表す記号: CDS

鎖の数: 一本鎖

存在位置: 1-207

トポロジー: 直鎖状

特徴を決定した方法: E

配列の種類: cDNA to genomic RNA

他の情報

起源

エンテロウイルス71型のVp4蛋白をコードする遺伝

生物名: enterovirus type 71 ★

子領域の1~207番目の塩基配列

配列

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21 22
 ATG GGT TCA CAA GTG TCT ACT CAG CGG
 TCC GGC TCC CAC GAG AAT TCT 48
 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
 5 10 15
 AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC AAC TAT 96
 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
 20 25 30
 TAC AAG GAC TCT TAT GCT GGC ACA GCA GGC AAG CAG ACC CTT AAA CAA 144
 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
 35 40 45
 GAC CCT GAC AAG TTT GCT AAT CCT GTC AAG GAC ATT TTC ACT GAA ATG 192
 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
 50 55 60
 GGC GCG CCA CTG AAG 207
 Ala Ala Pro Leu Lys
 65

【0049】配列番号: 7

配列の長さ: 207

配列の型: 核酸

鎖の数: 一本鎖

トポロジー: 直鎖状

配列の種類: cDNA to genomic RNA

起源

生物名: enterovirus type 71

* 株名: 0004/78

配列の特徴

特徴を表す記号: CDS

20 存在位置: 1-207

特徴を決定した方法: E

他の情報

エンテロウイルス71型のVp4蛋白をコードする遺伝

* 子領域の1~207番目の塩基配列

配列
 ATG GCA TCG CAG GTG TCC ACA CAA GGC TCT GGT TCG CAT GAA AAT TCT 48
 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
 5 10 15
 AAT TCA GGC ACT GAA GGT TCC ACT ATA AAC TAC ACC ACC ATC AAT TAC 96
 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
 20 25 30
 TAT AAG GAC TCT TAT GGC GCT ACA GCA GGC AAA CAG ACC CTT AAG CAA 144
 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
 35 40 45
 GAT CCA GAC AAG TTT GCA AAT CCC GTT AAA GAT ATT TTC ACT GAG ATG 192
 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
 50 55 60
 GCG GCA CCA CTG AAA 200
 7
 Ala Ala Pro Leu Lys
 65

【0050】配列番号: 8

配列の長さ: 207

配列の型: 核酸

鎖の数: 一本鎖

トポロジー: 直鎖状

配列の種類: cDNA to genomic RNA

起源

生物名: enterovirus type 71

* 株名: 3359/83

配列の特徴

特徴を表す記号: CDS

存在位置: 1-207

特徴を決定した方法: E

他の情報

エンテロウイルス71型のVp4蛋白をコードする遺伝

* 子領域の1~207番目の塩基配列

配列

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23 24

ATG GGT TCA CAA GTA TCC ACT CAG CGG TCC GGC TCC CAC GAG AAT TCT 48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser

5 10 15

AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACT ATC AAC TAT 96
Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr

20 25 30

TAC AAG GAC TCT TAT GCT GGC ACA GCA GGC AAA CAG AGC CTC AAA CAA 144
Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln

35 40 45

GAC CGC GAC AAG TTT GCT AAT GCT GTC AAG GAC ATT TTC ACT GAA ATG 192
Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met

50 55 60

GGG GCG CCG CTG AAG 207
Ala Ala Pro Leu Lys

65

【0051】配列番号：9

配列の長さ：207

配列の型：核酸

鎖の数：一本鎖

トポロジー：直鎖状

配列の種類：cDNA to genomic RNA

起源

生物名：enterovirus type 71

* 株名：4132/85

配列の特徴

特徴を表す記号：CDS

存在位置：1-207

20 特徴を決定した方法：E

他の情報

エンテロウイルス71型のVp4蛋白をコードする遺伝

* 子領域の1~207番目の塩基配列

配列

ATG GGC TCA CAA GTG TCT ACT CAG CGA TCC GGC TCC CAC GAG AAT TCC 48
Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser

5 10 15

ANT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC ANC TAT 96
Xaa Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Xaa Tyr

20 25 30

TAC ANA GNC TCT TAT GCT GCA ACA GCA GGC ANA CAG AGY CTT ANA CAA 144
Tyr Xaa Xaa Ser Tyr Ala Ala Thr Ala Gly Xaa Gln Ser Leu Xaa Gln

35 40 45

GNC CCT GAT AAG TKT GCT AAT GCT GTC AAG GAC ATT TTC ACT GAA ATG 192
Xaa Pro Asp Lys Xaa Ala Xaa Pro Val Lys Asp Ile Phe Thr Glu Met

50 55 60

GGC GCG CCA CTA AAA 207
Ala Ala Pro Leu Lys

65

【0052】配列番号：10

配列の長さ：207

配列の型：核酸

鎖の数：一本鎖

トポロジー：直鎖状

配列の種類：cDNA to genomic RNA

起源

生物名：enterovirus type 71

40※ 株名：T86236a

配列の特徴

特徴を表す記号：CDS

存在位置：1-207

特徴を決定した方法：E

他の情報

エンテロウイルス71型のVp4蛋白をコードする遺伝

* 子領域の1~207番目の塩基配列

配列

ATG GGT ACA CAA GTA TCC ACT CAG GGG TCC GGC TCC CAC GAG AAT TCT 48
Met Gly Thr Gln Val Ser Thr Gln Xaa Ser Gly Ser His Glu Asn Ser

| (14) | | 特開平8-173195 | |
|---|----|-------------|-----|
| 25 | 5 | 10 | 15 |
| AAT TCA GCT ACA GAA GGC TCC ACC ATT AAC TAC ACT ACT ATC AAC TAT | | | 96 |
| Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr | | | |
| 20 | 25 | 30 | |
| TAC AAG GAC TCT TAT GCT GCT ACA GCA GGC AAA CAG AGC CTC AAA CAA | | | 144 |
| Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln | | | |
| 35 | 40 | 45 | |
| GAC CCC GAC AAG TTT GCT AAT CCT GTC AAG GAC ATT TTT ACT GAA ATG | | | 192 |
| Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met | | | |
| 50 | 55 | 60 | |
| GCG GCG CCA CTG AAG | | | 207 |
| Ala Ala Pro Leu Lys | | | |
| 65 | | | |

【0053】配列番号：11

配列の長さ：207

配列の型：核酸

鎖の数：一本鎖

トポロジー：直鎖状

配列の種類：cDNA to genomic RNA

起源

生物名：enterovirus type 71

※株名：1096/86

配列の特徴

特徴を表す記号：CDS

存在位置：1-207

特徴を決定した方法：E

他の情報

20 エンテロウイルス71型のVp4蛋白をコードする遺伝

※ 子領域の1~207番目の塩基配列

配列

| | |
|---|-----|
| ATG GCG TCA CAG GTG TCC ACA CAA GCG TCC GGT TCG CAT GAA AAC TCT | 48 |
| Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser | |
| 5 10 15 | |
| AAC TCA GCT ACT GAG GCG TCC ACC ATA AAC TAT ACT ACC ATC AAT TAC | 96 |
| Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr | |
| 20 25 30 | |
| TAC AAG GAC TCC TAT GCG GCG ACA GCA GCG AAA CAG AGC CTT AAG CAG | 144 |
| Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln | |
| 35 40 45 | |
| GAT CCA GAT AAG TTT GCG AAT CCT GTC AAG GAT ATT TTC ACT GAA ATG | 192 |
| Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met | |
| 50 55 60 | |
| GCA GCG CCA CTA AAG | 207 |
| Ala Ala Pro Leu Lys | |
| 65 | |

【0054】配列番号：12

配列の長さ：207

配列の型：核酸

鎖の数：一本鎖

トポロジー：直鎖状

配列の種類：cDNA to genomic RNA

起源

生物名：enterovirus type 71

※株名：1091/89

配列の特徴

40 特徴を表す記号：CDS

存在位置：1-207

特徴を決定した方法：E

他の情報

エンテロウイルス71型のVp4蛋白をコードする遺伝

※ 子領域の1~207番目の塩基配列

配列

| | |
|---|----|
| ATG GGT TCA CAA GTG TCT GCT CAG CGA TCC GCG TCC CAC GAG AAT TCC | 48 |
| Met Gly Ser Gln Val Ser Ala Gln Arg Ser Gly Ser His Glu Asn Ser | |
| 5 10 15 | |
| AAT TCA GCT ACA GAA GGC TCC ACC ATT AAT TAC ACT ACC ATC AAC TAT | 96 |

(15)

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27 28

Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr

20 25 30

TAC AAA GAC TCT TAT GCT GCA ACA GCA GGC AAA CAG AGC CTC AAA CAA 144

Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln

35 40 45

GAC CCT GAT AAG TTT GCT AAC GCT GTC AAG GAT ATT TTC ACT GAA ATG 192

Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met

50 55 60

GCT GCG CCA CTG AAG 207

Ala Ala Pro Leu Lys

65

【0055】配列番号: 13

* 株名: 0872/89

配列の長さ: 207

配列の特徴

配列の型: 核酸

特徴を表す記号: CDS

鎖の数: 一本鎖

存在位置: 1-207

トポロジー: 直鎖状

特徴を決定した方法: E

配列の種類: cDNA to genomic RNA

他の情報

起源

エンテロウイルス71型のVp4蛋白をコードする遺伝

生物名: enterovirus type 71 *

子領域の1~207番目の塩基配列

配列

ATG GGC TCA CAG GTG TCC ACA CAA CGC

TCC GGT TCG CAT GAA AAC TCT 48

Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser

5 10 15

AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96

Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr

20 25 30

TAC AAG GAC TCC TAT GCT GGC ACA GCA GGC AAA CAG AGC CTT AAA CAG 144

Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln

35 40 45

GAT CCA GAT AAG TTT GCA AAT GCT GTC AAA GAT ATT TTC ACT GAA ATG 192

Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met

50 55 60

GCA GCG CCA CTA AAG 207

Ala Ala Pro Leu Lys

65

【0056】配列番号: 14

* 株名: 2587/89

配列の長さ: 207

配列の特徴

配列の型: 核酸

特徴を表す記号: CDS

鎖の数: 一本鎖

40 存在位置: 1-207

トポロジー: 直鎖状

特徴を決定した方法: E

配列の種類: cDNA to genomic RNA

他の情報

起源

エンテロウイルス71型のVp4蛋白をコードする遺伝

生物名: enterovirus type 71 *

子領域の1~207番目の塩基配列

配列

ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48

Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser

5 10 15

AAC TCA GCT ACT GAG GGT TCC ACC ATA AAC TAC ACT ACC ATT AAT TAC 96

Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr

49. 特徴を決定した方法: E.

(17)

特開平 8 -

31
 TAC AAA GAC TCT TAT GCT GCA ACA GCA GGC AAA CAG AGC CTC AAA CAA 144
 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
 35 40 45
 GAC CCT GAT AAG TTT GCT AAC CCT GTC AAG GAT ATT TTC ACT GAA ATG 192
 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
 50 55 60
 GCT GCG CCA CTG AAG 207
 Ala Ala Pro Leu Lys
 65

【0059】配列番号：17

10*株名：0375/90

配列の長さ：207

配列の特徴

配列の型：核酸

特徴を表す記号：CDS

鎖の数：一本鎖

存在位置：1-207

トポロジー：直鎖状

特徴を決定した方法：E

配列の種類：cDNA to genomic RNA

他の情報

起源

エンテロウイルス71型のVp4蛋白を

生物名：enterovirus type 71

* 子領域の1~207番目の塩基配列

配列

ATG GGC TCA CAG GTG TCC ACA CAA CGC TCC GGT TCG CAT GAA AAC TCT 48
 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
 5 10 15
 AAC TCA GCT ACT CAG GGT TCC ACC ATA AAC TAT ACC ACC ATT AAT TAC 96
 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
 20 25 30
 TAC AAG GAC TCC TAT GCT GGC ACA GCA GGC AAA CAG ACC CTT AAA CAG 144
 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
 35 40 45
 GAT CCA GAT AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192
 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
 50 55 60
 GCA GCG CCA CTA AAG 207
 Ala Ala Pro Leu Lys
 65

【0060】配列番号：18

※株名：2136/90

配列の長さ：207

配列の特徴

配列の型：核酸

特徴を表す記号：CDS

鎖の数：一本鎖

存在位置：1-207

トポロジー：直鎖状

特徴を決定した方法：E

配列の種類：cDNA to genomic RNA

他の情報

起源

40 エンテロウイルス71型のVp4蛋白を

(18)

特開平 8 -

33 34
 35 40 45
 GAT CCA GAT AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192
 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
 50 55 60
 GCA GCG CCA CTA AAG 207
 Ala Ala Pro Leu Lys
 65

【0061】配列番号：19

※株名：2398/90

配列の長さ：207

配列の特徴

配列の型：核酸

10 特徴を表す記号：CDS

鎖の数：一本鎖

存在位置：1-207

トポロジー：直鎖状

特徴を決定した方法：E

配列の種類：cDNA to genomic RNA

他の情報

起源

エンテロウイルス71型のVp4蛋白を

生物名：enterovirus type 71

※ 子領域の1~207番目の塩基配列

配列

ATG GGC TCA CAG GTG TCC ACA CAA GCG TCC GGC TCA CAT GAA AAC TCT 48
 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
 10
 AAC TCA GCT ACT GAG GGC TCC ACC ATA AAC TAC ACT ACT ATT AAT TAC 96
 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
 20 30
 TAC AAG GAC TCC TAT GGC GCT ACA GCA GGC AAA CAG AGC CTC AAG CAG 144
 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
 40
 GAT CCA GAT AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192
 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
 50 60
 GCA GCG CCA CTA AAG 207
 Ala Ala Pro Leu Lys
 65

【0062】配列番号：20

※株名：4094/90

配列の長さ：207

配列の特徴

配列の型：核酸

特徴を表す記号：CDS

鎖の数：一本鎖

存在位置：1-207

トポロジー：直鎖状

特徴を決定した方法：E

配列の種類：cDNA to genomic RNA

他の情報

起源

エンテロウイルス71型のVp4蛋白を

生物名：enterovirus type 71 ※

子領域の1~207番目の塩基配列

配列

(19)

特開平 8 -

35 36
 GAT CCA GAC AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192
 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
 50 55 60
 GCA GCG CCA CTA AAA 207
 Ala Ala Pro Leu Lys
 65

【0063】配列番号：21

※株名：0419/90

配列の長さ：207

配列の特徴

配列の型：核酸

特徴を表す記号：CDS

鎖の数：一本鎖

10 存在位置：1-207

トポロジー：直鎖状

特徴を決定した方法：E

配列の種類：cDNA to genomic RNA

他の情報

起源

エンテロウイルス71型のVp4蛋白を

生物名：enterovirus type 71

※ 子領域の1～207番目の塩基配列

配列

ATG GGC TCA CAG GTG TCC ACG CAA CGC TCC GGC TCG CAT GAA AAC TCT 48
 Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser
 5 10 15
 AAT TCA GCT ACT GAG GGC TCC ACC ATA AAC TAT ACC ACC ATT AAT TAT 96
 Asn Ser Ala Thr Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr
 20 25 30
 TAC AAG GAC TCC TAT GGC GGC ACA GCA GGC AAA CAG AGT CTT AAG CAG 144
 Tyr Lys Asp Ser Tyr Ala Ala Thr Ala Gly Lys Gln Ser Leu Lys Gln
 35 40 45
 GAT CCA GAC AAG TTT GCA AAT CCT GTC AAA GAT ATT TTC ACT GAA ATG 192
 Asp Pro Asp Lys Phe Ala Asn Pro Val Lys Asp Ile Phe Thr Glu Met
 50 55 60
 GCT GCG CCA CTA AAG 207
 7
 Ala Ala Pro Leu Lys
 65

【0064】配列番号：22

※株名：G19

配列の長さ：207

配列の特徴

配列の型：核酸

特徴を表す記号：CDS

鎖の数：一本鎖

存在位置：1-207

トポロジー：直鎖状

特徴を決定した方法：E

配列の種類：cDNA to genomic RNA

他の情報

起源

コクサッキーA群ウイルス16型のVp

生物名：enterovirus coxsackievirus A16

※ する遺伝子領域の1～207番目の塩基

配列

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37 38

Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met

50 55 60

GCT CCT CCC TTG AAA 207

Ala Pro Pro Leu Lys

65

【0065】配列番号: 23

* 株名: 1547/79

配列の長さ: 207

配列の特徴

配列の型: 核酸

特徴を表す記号: CDS

鎖の数: 一本鎖

存在位置: 1-207

トポロジー: 直鎖状

10 特徴を決定した方法: E

配列の種類: cDNA to genomic RNA

他の情報

起源

コクサッキーA群ウイルス16型のVp4蛋白をコード

生物名: coxsackievirus A16

* する遺伝子領域の1~207番目の塩基配列

配列

ATG GCG TCA CAG GTT TCC ACT CAG CGG TCT GCG TCA CAT GAG AAC TCA 48

Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser

5 10 15

AAC TCT GCA TCG GAG GGT TCA ACT ATA AAT TAT ACA ACC ATA AAT TAC 96

Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr

20 25 30

TAT AAG GAT GCA TAT GCT GCA AGT GCG GCG GCG CAG GAT ATG TCC CAA 144

Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln

35 40 45

GAC CCG AAG AAA TTT ACC GAT CCT GTT ATG GAC GTT ATA CAT GAG ATG 192

Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met

50 55 60

GCT CCA CCA CTT AAA 207

Ala Pro Pro Leu Lys

65

【0066】配列番号: 24

30※ 株名: 4057/81

配列の長さ: 207

配列の特徴

配列の型: 核酸

特徴を表す記号: CDS

鎖の数: 一本鎖

存在位置: 1-207

トポロジー: 直鎖状

特徴を決定した方法: E

配列の種類: cDNA to genomic RNA

他の情報

起源

コクサッキーA群ウイルス16型のVp4蛋白をコード

生物名: coxsackievirus A16

※ する遺伝子領域の1~207番目の塩基配列

配列

ATG GCG TCA CAG GTC TCC ACT CAG CGG TCT GCG TCA CAT GAG AAC TCA 48

Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser

5 10 15

AAC TCT GCA TCG GAG GGT TCA ACT ATA AAT TAC ACA ACC ATA AAT TAC 96

Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr

20 25 30

TAT AAG GAT GCA TAT GCT GCA AGT GCG GCG GCG CAG GAT ATG TCC CAA 144

Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln

35 40 45

GAC CCG AAG AAA TTT ACC GAT CCT GTC ATG GAC GTT ATA CAT GAG ATG 192

Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met

50 55 60

| | | |
|---|------------------------------|-------------|
| | (21) | 特開平8-173195 |
| 39 | | 40 |
| QCT CCA CCA CTC AAA | | 207 |
| Ala Pro Pro Leu Lys | | |
| 65 | | |
| 【0067】配列番号: 25 | * 株名: 0216/86 | |
| 配列の長さ: 207 | 配列の特徴 | |
| 配列の型: 核酸 | 特徴を表す記号: CDS | |
| 鎖の数: 一本鎖 | 存在位置: 1-207 | |
| トポロジー: 直鎖状 | 特徴を決定した方法: E | |
| 配列の塩類: cDNA to genomic RNA | 他の情報 | |
| 起源 | 10 コクサッキーA群ウイルス16型のVp4蛋白をコード | |
| 生物名: coxsackievirus A16 | * する遺伝子領域の1~207番目の塩基配列 | |
| 配列 | | |
| ATG GCG TCA CAG GTC TCC ACT CAG GCG TCT GCG TCA CAC GAA AAC TCA | 48 | |
| Met Gly Ser Gln Val Ser Thr Gln Xaa Ser Gly Ser His Glu Asn Ser | | |
| 5 10 15 | | |
| AAC TCT GYA TCG GCG GGT ACA WCT ATA AAT TAC ACA CCC ATA AAT TAC | 96 | |
| Asn Ser Xaa Ser Xaa Gly Thr Xaa Ile Asn Tyr Thr Pro Ile Asn Tyr | | |
| 20 25 30 | | |
| TAT AAG GAT CCA TAT GCT CCA AGT GCG GGA CGM CAG GAT ATG TCC CAG | 144 | |
| Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln | | |
| 35 40 45 | | |
| GAC CCG AAG AAA TTC ACC GAT GCT GTC ATG GAC GTT ATA CAT GAG ATG | 192 | |
| Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met | | |
| 50 55 60 | | |
| QCT CCA CCG CTC AAA | 207 | |
| Ala Pro Pro Leu Lys | | |
| 65 | | |
| 【0068】配列番号: 26 | * 株名: 0241/91 | |
| 配列の長さ: 207 | 配列の特徴 | |
| 配列の型: 核酸 | 30 特徴を表す記号: CDS | |
| 鎖の数: 一本鎖 | 存在位置: 1-207 | |
| トポロジー: 直鎖状 | 特徴を決定した方法: E | |
| 配列の塩類: cDNA to genomic RNA | 他の情報 | |
| 起源 | コクサッキーA群ウイルス16型のVp4蛋白をコード | |
| 生物名: coxsackievirus A16 | * する遺伝子領域の1~207番目の塩基配列 | |
| 配列 | | |
| ATG GCG TCA CAG GTC TCC ACT CAA CGG TCT GCG TCA CAT GAG AAC TCA | 48 | |
| Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser | | |
| 5 10 15 | | |
| AAC TCA GCA TCA GAG GGT TCA ACT ATA AAT TAC ACA ACC ATA AAT TAC | 96 | |
| Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr | | |
| 20 25 30 | | |
| TAT AAA GAT CCA TAT GCT GCG AGT GCG GCG CCG CAG GAT ATG TCC CAA | 144 | |
| Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln | | |
| 35 40 45 | | |
| GAT CCG AAG AAA TTT ACC GAT GCT GTT ATG GAT GTT ATA CAC GAG ATG | 192 | |
| Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met | | |
| 50 55 60 | | |
| QCT CCA CCA CTC AAA | 207 | |
| Ala Pro Pro Leu Lys | | |

| | | |
|---|------------------------------|-------------|
| | (21) | 特開平8-173195 |
| 39 | | 40 |
| QCT CCA CCA CTC AAA | | 207 |
| Ala Pro Pro Leu Lys | | |
| 65 | | |
| 【0067】配列番号: 25 | * 株名: 0216/86 | |
| 配列の長さ: 207 | 配列の特徴 | |
| 配列の型: 核酸 | 特徴を表す記号: CDS | |
| 鎖の数: 一本鎖 | 存在位置: 1-207 | |
| トポロジー: 直鎖状 | 特徴を決定した方法: E | |
| 配列の種別: cDNA to genomic RNA | 他の情報 | |
| 起源 | 10 コクサッキーA群ウイルス16型のVp4蛋白をコード | |
| 生物名: coxsackievirus A16 | * する遺伝子領域の1~207番目の塩基配列 | |
| 配列 | | |
| ATG GCG TCA CAG GTC TCC ACT CAG GCG TCT GCG TCA CAC GAA AAC TCA | 48 | |
| Met Gly Ser Gln Val Ser Thr Gln Xaa Ser Gly Ser His Glu Asn Ser | | |
| 5 10 15 | | |
| AAC TCT GYA TCG GCG GGT ACA WCT ATA AAT TAC ACA CCC ATA AAT TAC | 96 | |
| Asn Ser Xaa Ser Xaa Gly Thr Xaa Ile Asn Tyr Thr Pro Ile Asn Tyr | | |
| 20 25 30 | | |
| TAT AAG GAT CCA TAT GCT CCA AGT GCG GGA GGM CAG GAT ATG TCC CAG | 144 | |
| Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln | | |
| 35 40 45 | | |
| GAC CCG AAG AAA TTC ACC GAT CCT GTC ATG GAC GTT ATA CAT GAG ATG | 192 | |
| Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met | | |
| 50 55 60 | | |
| QCT CCA CCG CTC AAA | 207 | |
| Ala Pro Pro Leu Lys | | |
| 65 | | |
| 【0068】配列番号: 26 | * 株名: 0241/91 | |
| 配列の長さ: 207 | 配列の特徴 | |
| 配列の型: 核酸 | 30 特徴を表す記号: CDS | |
| 鎖の数: 一本鎖 | 存在位置: 1-207 | |
| トポロジー: 直鎖状 | 特徴を決定した方法: E | |
| 配列の種別: cDNA to genomic RNA | 他の情報 | |
| 起源 | コクサッキーA群ウイルス16型のVp4蛋白をコード | |
| 生物名: coxsackievirus A16 | * する遺伝子領域の1~207番目の塩基配列 | |
| 配列 | | |
| ATG GCG TCA CAG GTC TCC ACT CAA CCG TCT GCG TCA CAT GAG AAC TCA | 48 | |
| Met Gly Ser Gln Val Ser Thr Gln Arg Ser Gly Ser His Glu Asn Ser | | |
| 5 10 15 | | |
| AAC TCA CCA TCA GAG GGT TCA ACT ATA AAT TAC ACA ACC ATA AAT TAC | 96 | |
| Asn Ser Ala Ser Glu Gly Ser Thr Ile Asn Tyr Thr Thr Ile Asn Tyr | | |
| 20 25 30 | | |
| TAT AAA GAT CCA TAT GCT GCG AGT GCG GCG CCG CAG GAT ATG TCC CAA | 144 | |
| Tyr Lys Asp Ala Tyr Ala Ala Ser Ala Gly Arg Gln Asp Met Ser Gln | | |
| 35 40 45 | | |
| GAT CCG AAG AAA TTT ACC GAT CCT GTT ATG GAT GTT ATA CAC GAG ATG | 192 | |
| Asp Pro Lys Lys Phe Thr Asp Pro Val Met Asp Val Ile His Glu Met | | |
| 50 55 60 | | |
| QCT CCA CCA CTC AAA | 207 | |
| Ala Pro Pro Leu Lys | | |

| | | |
|---|---|------------------------------|
| <p>41</p> <p>65</p> | <p>(22)</p> | <p>特開平8-173195</p> <p>42</p> |
| <p>【0069】配列番号：27</p> <p>配列の長さ：16</p> <p>配列の型：核酸</p> <p>鎖の数：一本鎖</p> <p>トポロジー：直鎖状</p> | <p>* 配列の種類：他の核酸 合成DNA</p> <p>他の情報</p> <p>エンテロウイルスの5' 非翻訳領域の一部で antigenom ic sense RNAに相補性を有する配列</p> | |
| <p>配列</p> <p>CTACTTTGGG TGTCCG</p> | <p>*</p> | <p>16</p> |
| <p>【0070】配列番号：28</p> <p>配列の長さ：20</p> <p>配列の型：核酸</p> <p>鎖の数：一本鎖</p> <p>トポロジー：直鎖状</p> <p>配列の種類：他の核酸 合成DNA</p> | <p>* 配列の種類：他の核酸 合成DNA</p> <p>他の情報</p> <p>10 エンテロウイルスのV p 2 蛋白をコードする遺伝子領域 の一部で genomic sense RNAに相補性を有する配列。配 列中、塩基番号6番目のQはT又はCを示し、塩基番号 18番目のNはA又はC又はG又はTを示す。</p> | <p>16</p> |
| <p>配列</p> <p>GGTAAQTTC ACCAGCACC</p> | <p>※</p> | <p>20</p> |
| <p>【0071】配列番号：29</p> <p>配列の長さ：20</p> <p>配列の型：核酸</p> <p>鎖の数：一本鎖</p> <p>トポロジー：直鎖状</p> | <p>★ 配列の種類：他の核酸 合成DNA</p> <p>他の情報</p> <p>エンテロウイルスの5' 非翻訳領域の一部で antigenom ic sense RNAに相補性を有する配列</p> | <p>20</p> |
| <p>配列</p> <p>CATCTTTGGG TGTCCGTGT</p> | <p>★</p> | <p>20</p> |
| <p>【0072】配列番号：30</p> <p>配列の長さ：20</p> <p>配列の型：核酸</p> <p>鎖の数：一本鎖</p> <p>トポロジー：直鎖状</p> | <p>☆ 配列の種類：他の核酸 合成DNA</p> <p>他の情報</p> <p>エンテロウイルスのVP 2 蛋白をコードする遺伝子領域 の一部で genomic sense RNAに相補 性を有する配列</p> | <p>20</p> |
| <p>配列</p> <p>TCAGGCAACT TCCAGCACCA</p> | <p>◆</p> | <p>20</p> |
| <p>【0073】配列番号：31</p> <p>配列の長さ：20</p> <p>配列の型：核酸</p> <p>鎖の数：一本鎖</p> <p>トポロジー：直鎖状</p> | <p>◆ 配列の種類：他の核酸 合成DNA</p> <p>他の情報</p> <p>エンテロウイルスのV p 4 蛋白をコードする遺伝子領域 の118～137番目の antigenomic sense RNAに相補 性を有する配列</p> | <p>20</p> |
| <p>配列</p> <p>AGGCTCTGTT TGGCTGCTGT</p> | <p>◆</p> | <p>20</p> |
| <p>【0074】配列番号：32</p> <p>配列の長さ：20</p> <p>配列の型：核酸</p> <p>鎖の数：一本鎖</p> <p>トポロジー：直鎖状</p> | <p>* 配列の種類：他の核酸 合成DNA</p> <p>他の情報</p> <p>40 エンテロウイルスのV p 4 蛋白をコードする遺伝子領域 の118～137番目の antigenomic sense RNAに相補 性を有する配列</p> | <p>20</p> |
| <p>配列</p> <p>ATATCTCTGGC GCCCGCACT</p> | <p>*</p> | <p>20</p> |
| <p>【0075】配列番号：33</p> <p>配列の長さ：20</p> <p>配列の型：核酸</p> <p>鎖の数：一本鎖</p> <p>トポロジー：直鎖状</p> | <p>※ 配列の種類：他の核酸 合成DNA</p> <p>他の情報</p> <p>エンテロウイルスのV p 4 蛋白をコードする遺伝子領域 の122～141番目の antigenomic sense RNAに相補 性を有する配列</p> | <p>20</p> |

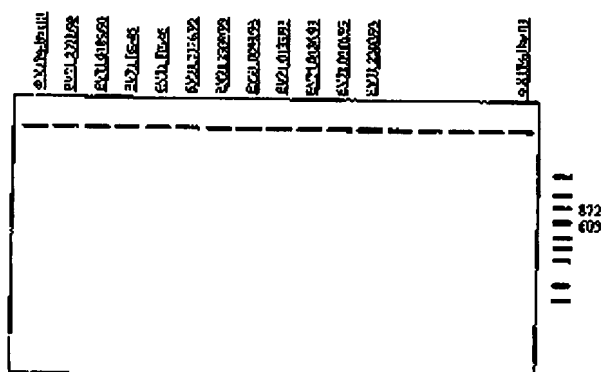
| | |
|-----------------------|---|
| 43 | 特開平8-173195 |
| TTTGAQCTC TGTTCQCTG | 44 |
| 【0076】配列番号：34 | 20 |
| 配列の長さ：20 | * 配列の種類：他の核酸 合成DNA |
| 配列の型：核酸 | 他の情報 |
| 鎖の数：一本鎖 | エンテロウイルスのVp4蛋白をコードする遺伝子領域の122～141番目の antigenomic sense RNAに相補性を有する配列 |
| トポロジー：直鎖状 | * 性 |
| 配列 | |
| GGACATATCC TGGGCCCCCG | 20 |
| 【0077】配列番号：35 | * 配列の種類：他の核酸 合成DNA |
| 配列の長さ：20 | 10 他の情報 |
| 配列の型：核酸 | エンテロウイルスのVp4蛋白をコードする遺伝子領域の181～200番目の antigenomic sense RNAに相補性を有する配列 |
| 鎖の数：一本鎖 | * 性 |
| トポロジー：直鎖状 | |
| 配列 | |
| GGCGCTGCCA TTTCAGTGAA | 20 |
| 【0078】配列番号：36 | ★ 配列の種類：他の核酸 合成DNA |
| 配列の長さ：20 | 他の情報 |
| 配列の型：核酸 | エンテロウイルスのVp4蛋白をコードする遺伝子の181～200番目の antigenomic sense RNAに相補性を有する配列 |
| 鎖の数：一本鎖 | ★20 有する配列 |
| トポロジー：直鎖状 | |
| 配列 | |
| GGTGGAGCCA TCTCATGTAT | 20 |
| 【0079】配列番号：37 | ☆ 他の情報 |
| 配列の長さ：20 | エンテロウイルスのVp4蛋白をコードする遺伝子領域の173～192番目の antigenomic sense RNAに相補性を有する配列。配列中、塩基番号19番目のQは、C又はTを示す。 |
| 配列の型：核酸 | ☆ |
| 鎖の数：一本鎖 | |
| トポロジー：直鎖状 | |
| 配列の種類：他の核酸 合成DNA | |
| 配列 | |
| CATTTCACTG AAAATRTCTT | 20 |
| 【0080】配列番号：38 | ◆ 配列の種類：他の核酸 合成DNA |
| 配列の長さ：20 | 他の情報 |
| 配列の型：核酸 | エンテロウイルスのVp4蛋白をコードする遺伝子領域の173～192番目の antigenomic sense RNAに相補性を有する配列 |
| 鎖の数：一本鎖 | ◆ 性 |
| トポロジー：直鎖状 | |
| 配列 | |
| CATCTCTGT ATAACRTCCA | 20 |
| 【0081】配列番号：39 | * 配列の種類：他の核酸 合成DNA |
| 配列の長さ：20 | 他の情報 |
| 配列の型：核酸 | 40 エンテロウイルスのVp4をコードする遺伝子領域の118～137番目の antigenomic sense RNAの相補性を有する配列 |
| 鎖の数：一本鎖 | * 性 |
| トポロジー：直鎖状 | |
| 配列 | |
| ATATCTTGAC GCCCAGCGCT | 20 |
| 【0082】配列番号：40 | 他の情報 |
| 配列の長さ：20 | エンテロウイルスのVp4をコードする遺伝子領域の172～191番目の antigenomic sense RNAの相補性を有する配列。配列中、塩基番号3番目のQは、C又はTを示す。 |
| 配列の型：核酸 | |
| 鎖の数：一本鎖 | |
| トポロジー：直鎖状 | |
| 配列の種類：他の核酸 合成DNA | 50 |

| | |
|---|---|
| <p>45</p> <p>配列</p> <p>ATGTCATGTA TAACRTCCAT</p> <p>【0083】配列番号：41</p> <p>配列の長さ：20</p> <p>配列の型：核酸</p> <p>鎖の数：一本鎖</p> <p>トポロジー：直鎖状</p> <p>配列の種類：他の核酸 合成DNA</p> | <p>(24)</p> <p>特開平8-173195</p> <p>46</p> <p>20</p> <p>* 他の情報</p> <p>エンテロウイルスのVp4をコードする遺伝子領域の172～191番目の antigenomic sense RNAの相補性を有する配列。配列中、塩基番号18番目のQは、C又はTを示す。</p> |
| <p>配列</p> <p>ATTTCAGTGA AAATRTCQTT</p> <p>【図面の簡単な説明】</p> <p>【図1】CA16特異プローブ（配列番号32、配列番号38及び配列番号39）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>【図2】CA16特異プローブ（配列番号32、配列番号38及び配列番号39）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>【図3】CA16特異プローブ（配列番号32、配列番号38及び配列番号39）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>【図4】CA16特異プローブ（配列番号32、配列番号38及び配列番号39）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>【図5】CA16特異プローブ（配列番号32、配列番号38及び配列番号39）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>【図6】CA16特異プローブ（配列番号32、配列番号38及び配列番号39）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>【図7】CA16特異プローブ（配列番号32、配列番号38及び配列番号39）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>【図8】CA16特異プローブ（配列番号32、配列番号38及び配列番号39）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>【図9】EV71特異DNAプローブ（配列番号31及び配列番号41）と増幅DNAとのハイブリッド形成を示す図である。</p> | <p>20</p> <p>【図10】EV71特異DNAプローブ（配列番号31及び配列番号41）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>【図11】EV71特異DNAプローブ（配列番号31及び配列番号41）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>【図12】EV71特異DNAプローブ（配列番号31及び配列番号41）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>20 【図13】EV71特異DNAプローブ（配列番号31及び配列番号41）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>【図14】EV71特異DNAプローブ（配列番号31及び配列番号41）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>【図15】EV71特異DNAプローブ（配列番号31及び配列番号41）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>【図16】EV71特異DNAプローブ（配列番号31及び配列番号41）と増幅DNAとのハイブリッド形成を示す図である。</p> <p>30 【図17】エンテロウイルスの増幅遺伝子領域の位置、増幅DNAの大きさ並びにCA16特異DNAプローブ及びEV71特異DNAプローブの結合位置を示す図である。図中2ケタの数字は配列番号、（ ）内の数字はVp4蛋白をコードする遺伝子領域内の位置、[]内の数字はDNA断片の大きさ（bp）を示す。</p> |

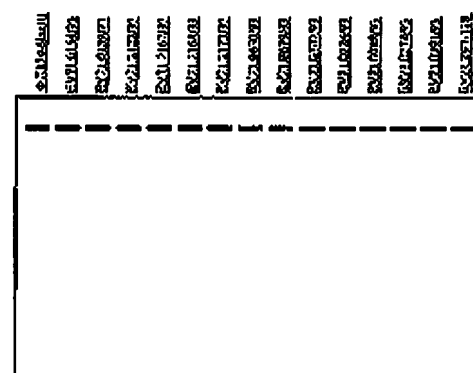
符号平 8 -

(26)

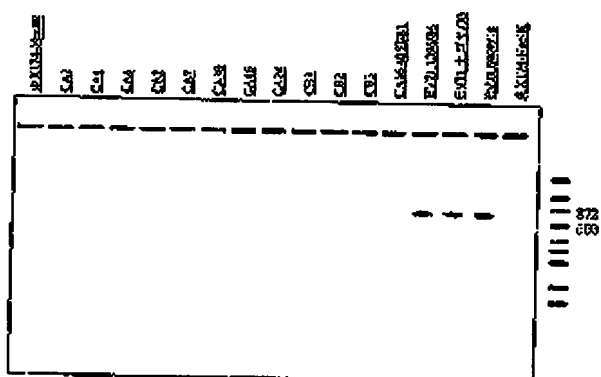
【図 7】



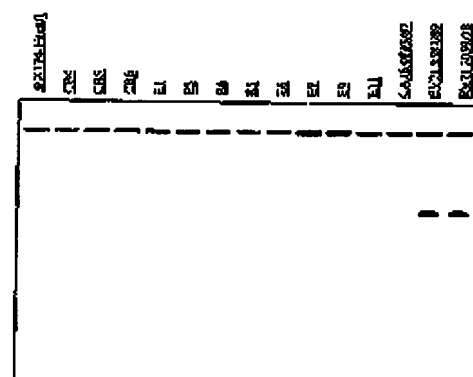
【図 8】



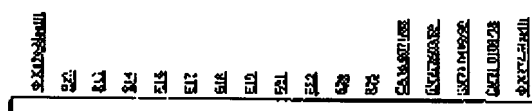
【図 9】



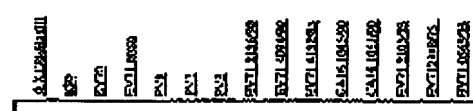
【図 10】



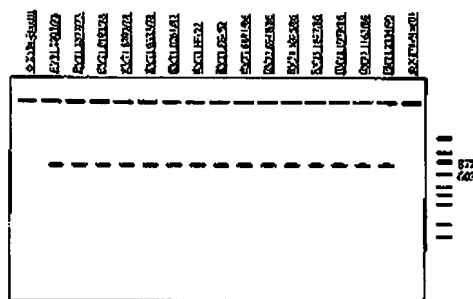
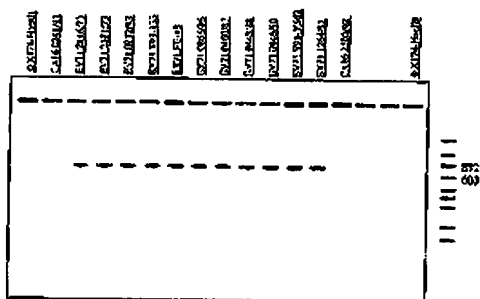
【図 11】



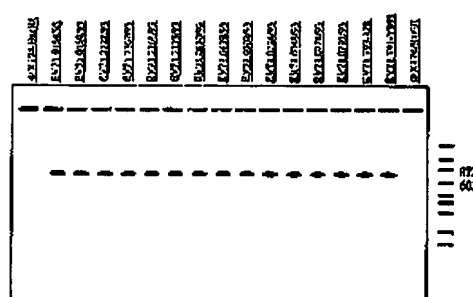
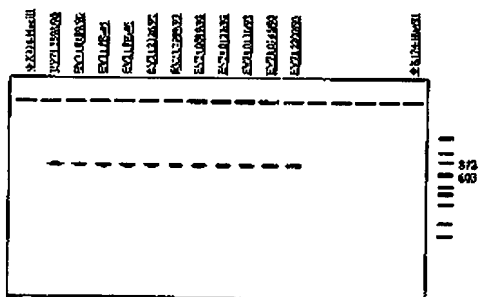
【図 12】



【图 14】



【图 16】



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【図 17】

